MYCOTAXON

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Xylaria nelumboniformis sp. nov.
(Ma, Vasilyeva† & Li— FIG. 1, p. 301)

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MYCOTAXON

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The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

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Errata from previous volumes

Volume 131(1)

p. iv, line 33
FOR: supported by morphological and sequence analyses
READ: supported by new morphological and sequence analyses

p. v, line 17
FOR: Records of terricolous lichens from Ecuador
READ: Records of terricolous lichens from páramos of southern Ecuador

p. v, line 28
FOR: *Bacidia, Micarea, Sagedia,* and *Stigmidium* spp.
READ: *Bacidia, Micarea, Sagedia,* and *Stigmidium* species

p. 88, line 29
FOR: ***E. sp.*** [2]
READ: ***E.* sp.** {2]

p. 93, line 23
FOR: Microbiol
READ: Microbiology

p. 153, line 13
FOR: Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad
READ: Departamento de Biología Vegetal I, Facultad de Biología, Universidad

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was issued on April 27, 2017

From the Editor-in-Chief

Our mycological river flows all too swiftly—Several days ago I reflected over my good fortune in serving 14 years as Mycotaxon Editor-in-Chief. One enjoyable benefit from editorial supervision is the opportunity to watch fledgling mycologists progress from first-time authors—most with no clue on how to formulate a rigorous research paper and lacking the techniques needed to produce an acceptable manuscript in English—to those who have mastered the basics but not yet how to publish a crisp valuable taxonomic treatise. Some either never progress beyond these beginning stages or (more likely) are defeated by the oft-insurmountable demands of simultaneously juggling teaching assignments, serious research, 'grantsmanship', family obligations, and gainful employment in mycology.

However, there are others who do persevere, progressing from 'top' student to newly hired assistant professor, eventually to establish their mycological credentials as both authors and valued (i.e., careful and helpful) expert reviewers. It is this last group that we value most and whose career paths we follow closely as they evolve from mycological neophytes to mycological masters. Eventually personal correspondence between editor and author develops into friendship (despite the fact that with increasing age, we editors tend to roar more exasperatedly over inattention to detail) and intense editorial interest in each author's achievements.

Alas, mycologists are not immortal, and early 2017 made fatal inroads into the ranks of our Great Mycologists. Late February brought the news of the unexpected death of Russian pyrenomycete specialist Larissa Vasilyeva in Vladivostok (two last papers are published posthumously in this issue with one plate serving as front cover). In early April we lost hyphomycete giants Walter Gams in Italy and Vadim Mel'nik in St. Petersburg, while early June delivered the sad news of the death of Brazil's oomycete and zygomycete specialist, José Ivanildo de Souza, in São Paulo.

The loss of four such important mycologists warrants more than a single sentence here. To that end, we include In Remembrance (p. xi–xix) on the following pages to provide photographs and brief biographies of four who gave so much to mycology.

Preparing the text (as always) facing Mycotaxon deadline, I appropriated material commemorating Mel'nik from Uwe Braun's thoughtful and warm April 10 publication in Schlechtendalia. The information is properly attributed, but I do hope that Uwe will forgive our reprinting one photo here without first asking permission.

We would like to think that this new section would not be needed again. Realistically, however, we expect to present such memoria in the future and so encourage readers and authors to notify us of deaths and send in short biographies and photographs for us to use as needed.

Mycotaxon 132(2) offers 23 research papers by 89 authors representing 13 countries and revised by 40 expert reviewers.

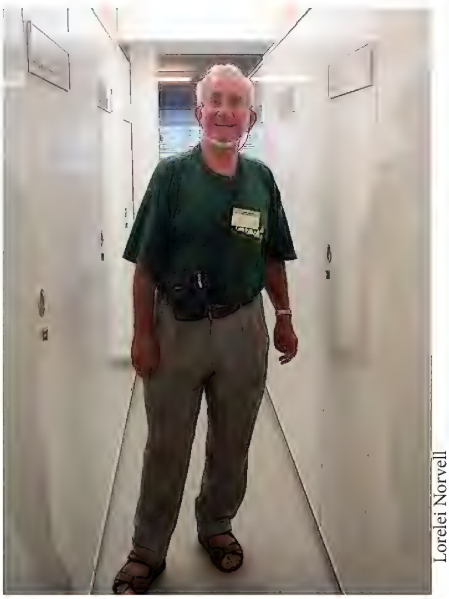
Within its pages are 3 new genera (*Arthrotaeniolella, Dictyotrichocladium,* and *Tretoheliocephala* from Brazil) and 15 species new to science representing *Arthrotaeniolella, Bharatheeya, Dictyotrichocladium, Minimelanolocus, Morganella, Pseudospiropes, Sporormiella,* and *Tretoheliocephala* from Brazil; *Corynespora* from India*; Elytroderma* from Russia*; Inocybe* from Pakistan*; Microidium* from Thailand; and *Neozygites, Pseudobaeospora,* and *Xylaria* from China.

Range extensions and/or new hosts for previously named taxa include new records of agarics (*Gymnopilus* in Thailand, *Inocybe* in Pakistan), lichens (*Lecidea* in China), macro- (8 new records in Mexico) and micro-ascomycetes (*Cephaliophora* in Poland, *Linkosia* & *Spadicoides* in China), stinkhorns (*Phallales* in Paraguay), and zygomycetes (*Cunninghamella,* including a key to the species found in Brazil). Additional information is presented for the bolete *Hourangia cheoi* from China and a full morphological reevaluation of the slime mould genus *Cribaria* in Mexico based on scanning electronic microscopy.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
8 July 2017

In remembrance



Lorelei Norvell

Walter Gams stands happily surrounded by anamorph collections in Vienna's Herbarium W at the end of the 2005 Nomenclature Section.

[Konrad] Walter Gams (1934–2017): Dr. Hab. (Prof.) Gams was born August 9, 1934, in Zurich, Switzerland, the son of bryologist and lichenologist Helmut Gams. He married Sophia Aaltine Luinge in 1943, was father to Hedwig and Mechthilde, and died unexpectedly at age 83 on April 9 in Bomarzo, Italy, only two weeks after depositing two *Phoma* cultures in the Centraalbureau voor Schimmelcultures (CBS), renamed Westerdijk Fungal Biodiversity Institute on 10 February 2017) in Utrecht, Netherlands.

After obtaining his PhD at Austria's University of Innsbruck in 1960, Walter joined CBS in 1967 as Senior Scientist and worked there until retiring as CBS Director in 1999 and as Senior Emeritus Scientist in 2007. Sometime in the 70s he founded the famed CBS summer course later attended by mycologists from all over the world. From 1972 onwards, he also served as Lecturer at Germany's Technology University in Aachen.

Gams was highly influential in fungal nomenclature, starting with the discussions surrounding sanctioning of names prior to the 1981 International Botanical Congress (IBC). Elected to the IBC Permanent Nomenclature Committee for Fungi in 1984, he succeeded Richard P. Korf as Secretary in 1991 and retired as NCF Secretary in 2005 and from the NCF in 2011. During this time Walter also chaired the MSA Nomenclature Committee (2001–2004).

A prolific author, with well over 200 publications to his credit, Walter began work on the zygomycete *Mortierella* before becoming a world-renowned expert in asexual (anamorphic) fungi. He teased apart and monographed the difficult speciose genera of *Acremonium, Fusarium, Trichoderma,* and *Verticillium* and participated in proposing over 33 generic names and 446 species epithets. MycoBank cites 671 names proposed by "W. Gams" and coauthors and lists three genera (*Gamsia, Gamsiella, Gamsylella*), 21 species, and one variety named after him.

His more important books include Fungi in agricultural soils (Domsch & al. 1972), Cephalosporium-artige Schimmelpilze (Hyphomycetes) (Gams 1971, with his 1972 defense securing for him the title of 'Dr. Hab.'), Compendium

Lorelei Norvell

XVII Intnernational Botanical Congress, Vienna 2005—Left: NCF Secretary Gams discusses why micromycetologists need illustrations as types during IBC Section recess on July 14. Right: On July 16 on the herbarium rooftop, Walter focuses his camera while Dr. Passauer enthusiastically names the artists atop the Kunst Museum overlooking Maria Theresa Platz and David Hawksworth walks along the parapet toward the Vienna Woods.

of Soil Fungi (Domsch & al. 1980), Supplement and Corrigendum to the Compendium of Soil Fungi (Gams 1983), CBS course of mycology (Gams & al. 1987), and The genera of hyphomycetes (Seifert & al. 2011).

An indefatigable teacher and mentor, Walter also served as Editor for Allionia, Cryptogamie-Mycologie, Mycologia Austriaca, Nova Hedwigia, Phytopathologia Mediterranea, Studies in Mycology, and Zeitschrift für Mykologie. He published 12 papers in Mycotaxon [including the controversial "A critical response to the Amsterdam Declaration (2011; 116: 501–512) where he and 78 other authors passionately defended preservation of dual nomenclature]. Walter also served Mycotaxon frequently (12 times since 2004) as an excellent and knowledgeable pre-submission expert reviewer.

An honorary member of the British Mycological Society and Mycological Society of America (MSA), Walter received MSA's highest award, Distinguished Mycologist, in 2005 and the Anton de Bary medal from the Deutschen Phytomedizinischen Gesellschaft in 2012. One letter nominating Dr. Gams as Distinguished Mycologist noted "Five personal characters and abilities mark a person as being distinguished and a cut above others. These are: passion for the work; excellence in its execution; creativity in approach; communication of research results; and service to science" (2006, Inoculum 56(5): 15). Walter epitomized all five.

I was fortunate to meet Walter personally over a glass of wine at the 1994 IMC7 in Vancouver BC. Knowing of my nerdish interest in fungal nomenclature, he invited me to attend a meeting of the IBC NCF the next morning. I was hooked sufficiently that he nominated me for membership on the committee, to which I was elected at the 1999 St. Louis IBC. Probably because I submitted opinions (well thought out or otherwise) on everything before his ballot deadlines, he later mentored my taking over as Secretary at the 2005 Vienna IBC Nomenclature Section, where he

Lorelei Norvell

Courtesy of Walter Gams

Left—Walter and David Hawksworth discuss changes in the Vienna Code over dessert and wine at the IBC XVII in Vienna. Right—MSA Distinguished Mycologist Gams would soon be soaked by boiling surf on the black sands of the 'Big Island' (Hawai'i) in 2006. Below—Walter stands on a summit in the Italian alps with Takamichi Orihara in June 2012.

and David Hawksworth maneuvered me through the sessions and Viennese streets and restaurants. While nomenclatural differences between opposing factions run deep (often vituperatively so), I was fortunate to witness first hand the rather stunning collegial amiability evinced by those two mycological nomenclatural giants at lunches and dinners, each glancing at me with twinkling eyes to see whether I 'got' a particular point contrary to the other's point of view.

After Vienna, Walter became a good friend and frequent correspondent. We met frequently at MSA, IMC, and IBC meetings and exchanged monthly Emails and photos. All of us lucky enough to receive his annual December PDFs became an integral and appreciative member of his far-flung mycological family. It may seem odd to regard death at 83 as being cut down in one's prime, but given Walter's peripatetic nature and innate vigor, he was. We will all miss him.

ResearchGate photo

Vadim Alexandrovich Mel'nik (1937–2017):

Note: The information below (and more) comes mainly from Uwe Braun's excellent personal tribute to his close friend and colleague (Schlechtendalia 32: 29–34, 2017).

Vadim Mel'nik was born in Daugavpils, Latvia, on March 16, 1937. After researching spruce canker in Kaliningrad forests, he graduated in 1960 as 'forestry engineer' from the Leningrad Forestry Academy and began post-graduate work at the Komarov Botanical Institute in 1962, where he obtained his PhD based on phytopathogenic asexual fungi of the Leningrad region and in 1986 received his final academic credentials in his defense of his research on coelomycetes of the former USSR. At the Komarov, Mel'nik advanced to Senior Research Fellow in 1977, was promoted to Leading Researcher (Laboratory of Systematics and Geography of Fungi) in 1998, and served as Curator of the fungal herbarium LE for 15 years.

Throughout his career, Mel'nik collected widely throughout the former Soviet Union. A highly esteemed hyphomycete and coelomycete specialist, he also identified "countless fungal samples collected by colleagues from all over the world" and was frequently invited to speak at symposia and important mycological meetings in other countries. Toward the end of his life, he concentrated on microfungi collected during a research program by the Vietnam-Russian Tropical Research & Technology Centre for which he published numerous papers and several new species.

Among more than 220 scientific publications, his most important publications include his 1977 *Ascochyta* monograph and its translation, Key to the fungi of the genus *Ascochyta* Lib. (*Coelomycetes*) (Mel'nik 2000), Imperfect fungi on species of trees and shrubs (Mel'nik & Popushoi 1992), Cercosporoid fungi from Russia and adjacent countries (Braun & Mel'nik 1997), and two volumes covering the coelomycetes and dematiaceous hyphomycetes of Russia (Mel'nik 1997, 2000). ResearchGate (https://www.researchgate.net/profile/Vadim_Melnik) provides additional information on 52 Mel'nik research publications.

Mel'nik was also a "gifted editor and proofreader" who reviewed manuscripts for numerous mycological journals (including Mycotaxon) and served on the editorial boards of Fungal Diversity, Fungal Diversity Research Series, Mycologia Balcanica/Mycobiota, and Mycosphere. He supported Mikologiya i Fitopatologiya from its inception in 1967 and also served as Editor or Editor-in-Chief for numerous Russian book projects and series.

Only a few Russian mycologists have received the Russian A.A. Yachevky medal awarded to Vadim Mel'nik in 2012. Two genera (*Melnikomyces, Melnikia*) and four species have been named in his honor, while he introduced "123 taxonomic novelties, most of them new species, including three new families and three new genera" (Braun 2017; MycoBank lists ~191 taxonomic names).

Reprinted from Braun (2017)

Vadim Mel'nik surrounded by pointsettias in Shangxi, China in 2014

Prof. Braun concludes his tribute with: "A long-standing friendship, over decades, defying all kinds of social and political changes, has been suddenly interrupted. I will painfully miss him, his unselfish generosity, always ready to help, his open-minded character, and his regular phone calls.... If there would be a 'Walk of Fame' for mycologists, he undoubtedly deserved his own star.... He will be remembered by all as not only a gifted mycologist but also someone who was always prepared to help and train others. Vadim Mel'nik lost his wife early to cancer. He is survived by his daughter, Irina, and grandchildren."

Dr. José Ivanildo de Souza [1973–2017]: We received the sad news of the death at the age of 44 of this irreplaceable young mycologist only three weeks ago. Dr. José Ivanildo graduated in the biological sciences from São Judas Tadeu University in 1995, where he focused on the taxonomy, ecology, and phylogeny of fungi (*Mucorales*) and oomycetes (*Oomycota*). He received his masters (2001) and PhD (2006) from Paulista State University Júlio de Mesquita Filho (UNESP) and served his post-doctorate at the Center for Parasitological and Vector Studies (CONICET, UNLP) in Argentina in 2011. At the time of his death José Ivanildo was Scientific Researcher, Director of Technical Services in Nuclear Research in Mycology, at the Instituto de Botânica in São Paulo.

Courtesy of André Cabral Santiago

José Ivanildo de Souza at a forest overlook in Brazil in 2014

"J.I. Souza" is cited in MycoBank as author or coauthor of five taxonomic names. He was a former editor of Check List and Brazilian Journal of Botany and served as expert peer reviewer for Mycology, Mycotaxon, Sydowia, Brazilian Journal of Botany, Hoehnea, and Nova Química. As senior author for Mycotaxon he published a 2007 paper on selected mucoralean fungi from soils contaminated with toxic metals and "Zygomycetes from the Reserva Biológica de Mogi Guau" (2011). A list of José Ivanildo's 29 research publications and other mycological contributions may be accessed on (https://www.researchgate.net/profile/Jose_De_Souza). Those who understand Portuguese will enjoy his "Get to know all about fungi" interview (https://www.youtube.com/watch?v=8UZzPMGWdvI) recorded by Todo Seu in 2013.

Dr. André Cabral Santiago (Federal University of Pernambuco), who shared the above photograph of his colleague, wrote: "Dr. José Ivanildo de Souza [1973–2017] was a renowned Brazilian mycologist who dedicated his professional life to the study of zygosporic fungi, contributing greatly to the knowledge of the mucoralean diversity in Brazil's Atlantic Forest and Cerrado regions in Brazil. In recent years he published several important manuscripts on zoosporic fungi. He worked as a researcher at the Institute of Botany of São Paulo and died as a consequence of a pancreatic cancer on June 5, 2017. During the time he worked as a mycologist, Dr. José Ivanildo inspired several mycologists to follow in his footsteps. He was a professional who always showed humility, ethics, and professionalism in his relationship with other researchers and students."

Larissa Nikolaevna Vasilyeva [1950–2017]. Larissa Vasilyeva (also transliterated as Larisa and Vasiljeva, Vassiljeva, or Vassilieva based on different linguistic conventions) was born in Kursk, western Russia. After graduating from Leningrad State University in 1972, she moved to Vladivostok, where she mastered pyrenomycetes while at the Far East Branch of the Russian Academy of Sciences Institute of Biology and Soil Science. Her PhD dissertation, Pyrenomycetes of the Russian Far East (1992), established her as an expert in the field, and MycoBank cites ~458 taxa (not including the two new species published in this issue) authored or coauthored by "Lar.N. Vassiljeva". In addition to her many pyrenomycete works, Dr. Vasilyeva wrote several philosophical treatises on general taxonomic theory—Systematics in Mycology (1990), Platonism in Systematics (1992)—and coauthored the popular Macrofungi associated with oaks of eastern North America (2008).

Dr. Vasilyeva was active throughout Asia and North America, serving several terms on the IMA Asian Mycology Committee and as member of the Mycological Society of Japan, Mycological Society of America, and the Russian Academy of Sciences. Her memorial in the Big Thicket Bulletin notes that Larissa "loved to collect fungi, wandering through the woods humming softly to herself. She specialized in very small ascomycetes ... partly, perhaps, because she had myopia or near-sightedness that allowed her to see clearly the small black dots of pyrenomycetes scattered along a dead branch. She would pick up a likely substrate, pull off her thick-lensed glasses and examine the stick without a hand lens because, basically, she had a "built-in" hand lens for eyes. In addition, she had a sense of the biology of these

Haixia Wu

Larissa Vasilyeva on Changbaoi Mountain (Jilin Province, northern China) in 2013

Larissa Vasilyeva in tropical Hainan Province (southern China) in 2013

fungi and thus knew where to look for them. She was fearless in her ability to endure hot and cold weather in search of her beloved pyrenomycetes." (Accessed 7 July 2017: http://www.thicketofdiversity.org/blog/tod-researcher-larissa-vassiljeva/)

Dr. Steve Stephenson (University of Arkansas) frequently collaborated with Larissa during her exploration of the "Asa Gray disjunction" that demonstrated that pyrenomycetes also produce closely related organisms in eastern North America and eastern Asia, but nowhere else in the world. He noted Vasilyeva's observation, "In some cases, morphologically similar fungi present in both regions and considered to represent the same species may instead be two distinct species. In other cases, a single species not recognized as such may occur in both regions."

My own encounters with this formidable scholar with the gentle soul began in 2000 with Larissa's desire to publish 'Further comments on the 'PhyloCode' (Inoculum 51(6): 5–7) to add to an earlier Scott Redhead commentary (Inoculum 51(3): 1–3). She concluded, "In general, I do not think that such word combinations as 'phylogenetic nomenclature' or 'PhyloCode' are well chosen. If taxonomists are to be able to subordinate characters properly and ... to circumscribe more natural groups, the taxon names (nomenclature) will automatically be 'phylogenetic'. As for the Code, it calls for the ordering of taxonomists, not taxa. If two taxonomists in Europe and Asia describe the same taxon, there really should be rules coordinating what that taxon can be called."

We exchanged several Emails while I 'polished' her English (my Russian training coming to our rescue) and simplified her complex thoughts on taxonomy and nomenclature for MSA readers. Our amiable electronic friendship resumed in 2004 with Mycotaxon, for which she contributed to 19 papers (including two published posthumously in 132-2) and thoroughly reviewed 11 others. We met all too briefly at Edinburgh's 2010 IMC, memorialized by the blurry photo at left. She tactfully returned the much lovelier one on p. xviii, taken by Haixia Ma in 2013. Although we had both been diagnosed with the same cancer, Larissa's 2016 therapy failed her, and she left us far too soon on February 23, only one week after her 67th birthday.

Lorelei Norvell

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Three species of *Linkosia* and *Spadicoides* new to China

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Abstract—Specimens of *Linkosia longirostrata*, *Spadicoides klotzschii*, and *S. sylvatica* were collected on dead branches of unidentified plants and identified as new records from China. They are described and illustrated based on the Chinese material. Keys are also provided to *Linkosia* and *Spadicoides* species recorded from China.

Key words—asexual morph, hyphomycetes, taxonomy

Introduction

Linkosia A. Hern.-Gut. & B. Sutton, established in 1997 with *Sporidesmium coccothrinacis* A. Hern.-Gut. & J. Mena [≡ *L. coccothrinacis* (A. Hern.-Gut. & J. Mena) A. Hern.-Gut. & B. Sutton] as type species, is mainly characterized by conidiophores that are reduced to monoblastic conidiogenous cells producing solitary, distoseptate conidia (Hernández-Gutiérrez & Sutton 1997, Wu & Zhuang 2005). After Zhang et al. (2009) provided a key to the then-known six species, six additional species were described by Ma et al. (2011), Almeida et al. (2014), Delgado (2014), and Conceição et al. (2016). Of the twelve species currently accepted in the genus (Conceição et al. 2016), five have been recorded from China (Wu & Zhuang 2005, Zhang et al. 2009, Ma et al. 2011).

Spadicoides S. Hughes, established in 1958 with *Helminthosporium binum* Corda [≡ *S. bina* (Corda) S. Hughes] as type species, is diagnosed by distinct, unbranched or sparingly branched conidiophores with integrated, polytretic, terminal or intercalary conidiogenous cells producing solitary, acropleurogenus, unicellular or euseptate conidia (Hughes 1958, Ellis 1971, Sinclair et al. 1985, Seifert et al. 2011). Forty-two species have been described, of which fifteen have

been recorded from China (Zhou et al. 1999, Lu et al. 2000, Ho et al. 2002, Wong et al. 2002, Cai et al. 2004, Li et al. 2010, Ma et al. 2010, 2012, 2014, 2016, Xia et al. 2014). Goh & Hyde (1996) and Ma et al. (2016) have provided synoptic tables of relevant morphological characters of *Spadicoides* species.

During our continuing surveys of saprobic microfungi from the forests of southern China, we have collected specimens representing three new Chinese records of *Linkosia* and *Spadicoides* on dead branches. They are described and illustrated below. The specimens are conserved in the Herbarium of the Department of Plant Pathology, Jiangxi Agricultural University, Nanchang, China (HJAUP).

Linkosia longirostrata G. Delgado, Mycotaxon 129(1): 42, 2014. FIG. 1

CONIDIOPHORES absent or very reduced, erect, 1-septate, dark brown, 21–35 µm long, 6–12.5 µm wide at the swollen base. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, lageniform or subcylindrical, smooth, brown to dark brown, determinate or occasionally percurrent, 15–27 × 5–6 µm, 3–4 µm wide at the truncate apex. CONIDIA holoblastic, solitary, acrogenous,

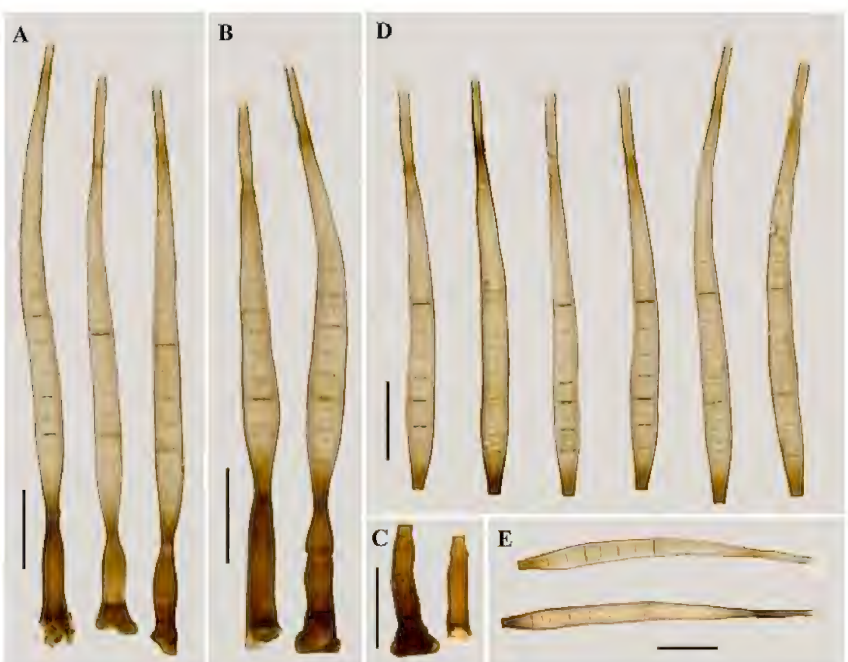

FIG. 1. *Linkosia longirostrata* (HJAUP M0162). A, B. Conidiophores, conidiogenous cells and conidia; C. Conidiophores and conidiogenous cells; D, E. Conidia. Scale bars = 20 µm.

straight or curved, obclavate to obclavate-rostrate, pale brown, smooth, 10–18-distoseptate, 82–126 µm long, 6.5–8.5 µm wide in the broadest part, 3–3.5 µm wide at the truncate base, apex extended into a pale brown to brown rostrum, 2–2.5 µm wide, and invested in 1–3 spherical or subspherical, hyaline or subhyaline mucous tunicae ca 6–15 µm diam.

Specimen examined: **CHINA**, **Jiangxi Province**, Lushan Mountain, on dead branches of an unidentified broadleaf tree, 7 November 2014, J. Ma (HJAUP M0162).

Comments – Delgado (2014) recorded this fungus on rachides of dead leaves from the palm species *Acoelorrhaphe wrightii* in Florida, USA, and compared it with similar species, including *Linkosia coccothrinacis*, *L. ponapensis* (Matsush.) R.F. Castañeda et al., *L. refugia* (B. Sutton & Pascoe) D.A.C. Almeida & Gusmão, and *L. canescens* (B. Sutton & Pascoe) D.A.C. Almeida & Gusmão. This is the first report of *L. longirostrata* in China. Our specimen is similar to the holotype from USA, but the holotype has smooth or slightly verrucose conidiogenous cells, and finely rough conidia without mucous tunicae at the apex (Delgado 2014); also, our specimen was collected from dicotyledenous wood, whereas the holotype was collected from monocotyledonous rachides.

Key to species of *Linkosia* from China

1. Conidia with 27–30 distosepta, 300–380 × 12–14 µm ..... *L. multisepta*
1. Conidia with <21 distosepta and <210 µm long 2
2. Conidia 12–16 µm or 13–15 µm wide 3
2. Conidia <12 µm wide 4
3. Conidia with mucilaginous sheath at the apex, 100–160 × 13–15 µm .. *L. fusiformis*
3. Conidia lacking mucilaginous sheath, 122–177 × 12–16 µm ..... *L. mori*
4. Conidia with 16–21 distosepta, 160–210 × 7.5–9.5 µm ..... *L. hibisci*
4. Conidia <126 µm long 5
5. Conidia with 12–14 distosepta, 110–125 × 9–12 µm ..... *L. obclavata*
5. Conidia with 10–18 distosepta, 82–126 × 6.5–8.5 µm ..... *L. longirostrata*

Spadicoides klotzschii S. Hughes, Fungi Canadenses: no. 8, 1973. Fig. 2

Conidiophores macronematous, mononematous, unbranched, erect, straight or flexuous, cylindrical, smooth, brown to dark brown, 5–8-septate, 85–135 × 3.5–4.5 µm. Conidiogenous cells polytretic, integrated, terminal and intercalary, cylindrical, smooth, brown, 6–11.5 × 2.5–3.5 µm. Conidia acropleurogenous, solitary, dry, obovoid, smooth, brown to dark brown, basal cell paler, 2-euseptate, 10–11.5 µm long, 5–6 µm wide in the broadest part, 0.5–1 µm wide at the base.

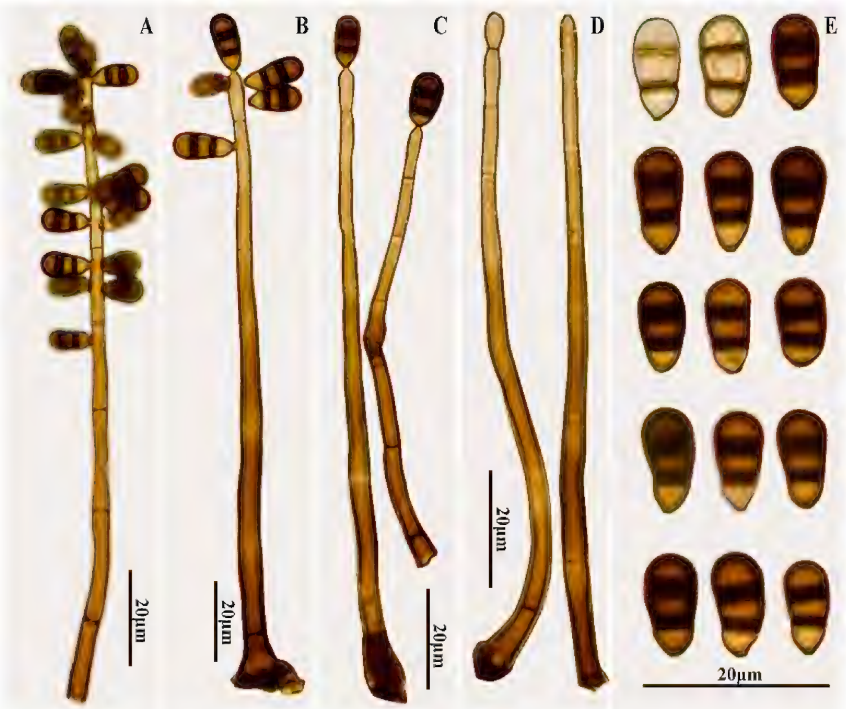

Fig. 2. *Spadicoides klotzschii* (HJAUP M0037). A–C. Conidiophores, conidiogenous cells and conidia; D. Conidiophores showing conidiogenous pores; E. Conidia. Scale bars = 20 μm.

Specimen examined: **CHINA**, **Guizhou Province**, Leigongshan National Nature Reserve, on dead branches of an unidentified broadleaf tree, 7 September 2013, J. Ma (HJAUP M0037).

Comments – Hughes (1973), who reported *Spadicoides klotzschii* on dead wood of *Alnus* and from unidentified hosts in British Columbia, Canada, compared it with *S. obovata* (Cooke & Ellis) S. Hughes. Subsequently, Pinruan et al. (2007) recorded this fungus from Thailand. Compared with our specimen, the holotype has slightly narrower conidia (4–5.5 μm) and longer conidiophores (55–220 μm; Hughes 1973). This is the first report of *S. klotzschii* in China.

Spadicoides sylvatica Heredia, R.F. Castañeda & R.M. Arias, Mycotaxon 130(2): 512, 2015. Fig. 3

Conidiophores macronematous, mononematous, unbranched, erect, straight or flexuous, cylindrical, determinate or occasionally percurrent,

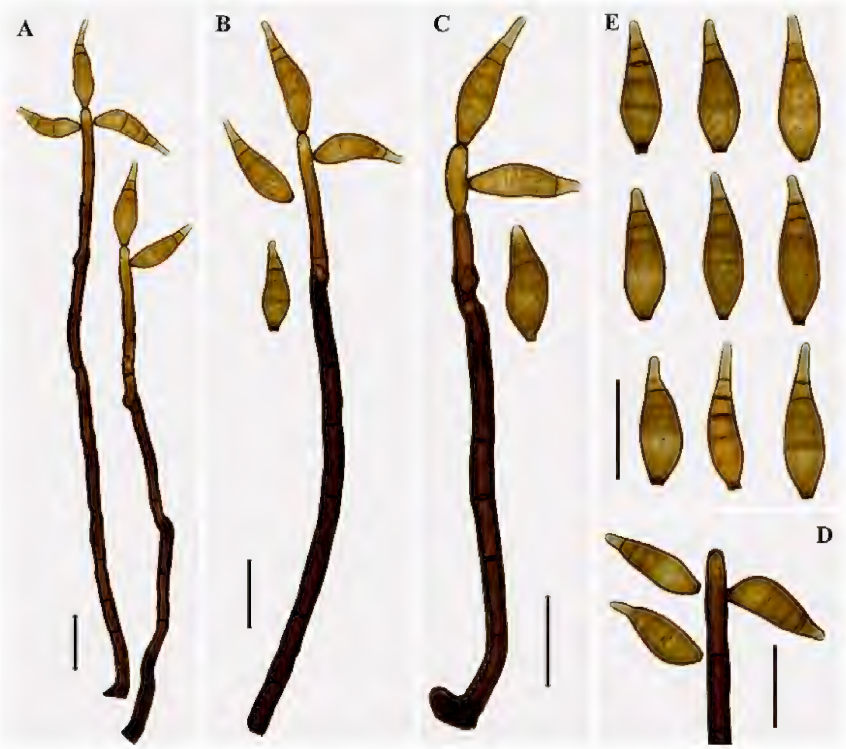

Fig. 3. *Spadicoides sylvatica* (HJAUP M0112). A–C. Conidiophores, conidiogenous cells and conidia; D. Conidiophore apex showing conidiogenous pores; E. Conidia. Scale bars = 20 μm.

smooth, dark brown, 6–13-septate, 130–220 × 4–7 μm. Conidiogenous cells polytretic, integrated, terminal and intercalary, cylindrical, smooth, brown, 14–25 × 3.5–5 μm. Conidia acropleurogenous, solitary, dry, ovate to obclavate, gradually tapered to an obtuse apex, smooth, brown, apical cell paler, (3–)4-euseptate, 25.5–35.5 × 6.5–9.5 μm, 1.5–2.5 μm wide at the base, 1.5–2.5 μm wide near apex.

Specimens examined: **China**, **Guangdong Province**, Liuxihe National Forest Park, on dead branches of an unidentified broadleaf tree, 10 July 2014, J. Ma (HJAUP M0112, HJAUP M0116).

Comments – *Spadicoides sylvatica* was described from Mexico and compared with *S. yunnanensis* L.G. Ma & X.G. Zhang (Heredia et al. 2015); it has not previously been recorded from China. Our collection corresponds well with the original description of *S. sylvatica* except that the holotype has slightly smaller conidia (22–32 × 7–8 μm) with verruculose apical cells (Heredia et al. 2015).

Key to species of *Spadicoides* from China

1. Conidia non-septate 2
1. Conidia septate 4
2. Producing a synanamorph predominantly with 2-septate synconidia .. *S. wufengensis*
2. Lacking a synanamorph 3
3. Conidia smooth, 4–6.5 × 3–4 µm ..... *S. atra*
3. Conidia verruculose, 11–18 × 4–6 µm ..... *S. arengae*
4. Conidia with both eusepta and distosepta 5
4. Conidia with only eusepta 6
5. Conidia either 0–1-euseptate, or with 1 euseptum + 1 distoseptum, 9–13 × 5–8 µm ..... *S. hodgkissii*
5. Conidia with 2 eusepta + 1 distoseptum, 13–14 × 5.5–6 µm ..... *S. versiseptatis*
6. Conidia verruculose 7
6. Conidia smooth 8
7. Conidia 5–7-euseptate, 30–72.5 × 5–7.5 µm ..... *S. bambusicola*
7. Conidia 9–12-euseptate, 98–166 × 8.5–12 µm ..... *S. bawanglingensis*
8. Conidia versicolorous 9
8. Conidia concolorous 12
9. Conidia 3–4-euseptate, 25.5–35.5 × 6.5–9.5 µm ..... *S. sylvatica*
9. Conidia predominantly 2-euseptate 10
10. Conidia 10–11.5 × 5–6 µm ..... *S. klotzschii*
10. Conidia at least 12.5 × 6 µm 11
11. Conidia ovoid, 15–22 × 7–10 µm ..... *S. camelliae*
11. Conidia obovate, clavate, 12.5–16 × 6–8.5 µm ..... *S. obovata*
12. Conidia bacilliform, 3-euseptate, 33–55 × 5.5–6.5 µm ..... *S. bacilliformis*
12. Conidia obclavate, oval to reniform or obpyriform to ovoid 3
13. Conidia 11–15-euseptate, 65–115 × 11.5–13 µm ..... *S. multiseptata*
13. Conidia <8-euseptate and <65 µm long 14
14. Conidia 2–3-euseptate, <28 µm long, rounded or acute at the apex 15
14. Conidia at least 5-euseptate, 40–65 µm long, rostrate 16
15. Conidia oval to reniform, 16–23 × 6.5–8 µm ..... *S. longchiensis*
15. Conidia obpyriform to ovoid, 18.5–28 × 6.5–10 µm ..... *S. yunnanensis*
16. Conidia 6–8-euseptate, 40–65 × 4.5–5.5 µm ..... *S. lushanensis*
16. Conidia 5(–6)-euseptate, 40–65 × 10.5–12.5 µm ..... *S. rostrata*

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Ascomycetous fungi of Siberia. 3. *Elytroderma baikalense* sp. nov.

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Abstract—*Elytroderma baikalense* is described herein as a species new to science, based on material collected from dying needles of *Pinus sylvestris* in the Lake Baikal region of Siberia. Images of ascomata, asci and ascospores are provided.

Key words—*Ascomycota*, pine disease, *Rhytismataceae*, taxonomy

Introduction

This paper is the third report on the ascomycetous fungi from the Lake Baikal region of Siberia (Morozova & Vasilyeva 2003; Vasilyeva & Morozova 2004). In 1995, a species of *Elytroderma* was found on second-year needles of *Pinus sylvestris* L. on Olkhon Island in Pribaikalsky National Park, Lake Baikal, and identified as *E. deformans* (Weir) Darker (Morozova 1996). However, additional study indicated that *Elytroderma deformans* occurs in North America, mostly on members of *Pinus* sect. *Trifoliae* (American hard pines, including *P. attenuata*, *P. banksiana*, *P. contorta*, *P. echinata*, *P. jeffreyi*, and *P. ponderosa*), but also on members of *Pinus* sect. *Parrya* (*P. cembroides* and *P. edulis*) (Hiratsuka 1987, Stone 1997). The range of *E. deformans* encompasses a major part of Canada

(British Columbia, the Northwest Territories, and Ontario) as well as the western United States (Arizona, California, Colorado, Idaho, Montana, Oregon, and Washington) (Minter 1980).

A second species, *Elytroderma torres-juanii* Diam. & Minter, was described on *Pinus brutia* Ten. from Greece; earlier names based on Spanish material—"*Hypoderma hispanicum*" J. Torres (Martínez & Torres 1965) and "*Elytroderma hispanicum*" (J. Torres) Darker (Darker 1967)—were invalid because no type specimen was indicated (Diamandis & Minter 1979). Later, *E. torres-juanii* was found on *Pinus stankewiczii* [= *P. brutia* var. *pityusa*] and *P. thunbergii* in the Crimea (Minter & Dudka 1996), and its overall distribution was characterized as "known on various species of pines from parts of Europe with a Mediterranean climate" (Minter: http://www.cybertruffle.org.uk/papers/rhytukra/elytrode.htm).

The European pines recorded as hosts of *Elytroderma torres-juanii* are in *Pinus* sect. *Pinus*, together with *P. sylvestris*, the host of the fungus from the Lake Baikal region. Thus, *E. torres-juanii* and the Siberian *Elytroderma* have hosts from the same section of *Pinus*. However, from a biogeographical standpoint, *E. deformans* would seem to be more likely to occur in eastern Siberia, with a distribution similar to that of *Delphinella balsameae* (Waterman) E. Müll., which has been reported from northeastern North America and also found in the Irkutsk region (Morozova & Vasilyeva 2003). This distribution does not apply to the Siberian *Elytroderma*, which differs from the two previously described species in ascospore characters.

Materials & methods

The specimens considered herein were collected by T.I. Morozova on Olkhon Island in Lake Baikal in 1995 and 1998. The specimens were studied microscopically using standard techniques. Images of the ascomata were obtained by T. I. Morozova using a Stereo Discovery V12 microscope equipped with an AxioCam ERc 5s camera, and images of asci and ascospores were obtained with an AxioLab A1 microscope and the same type of camera.

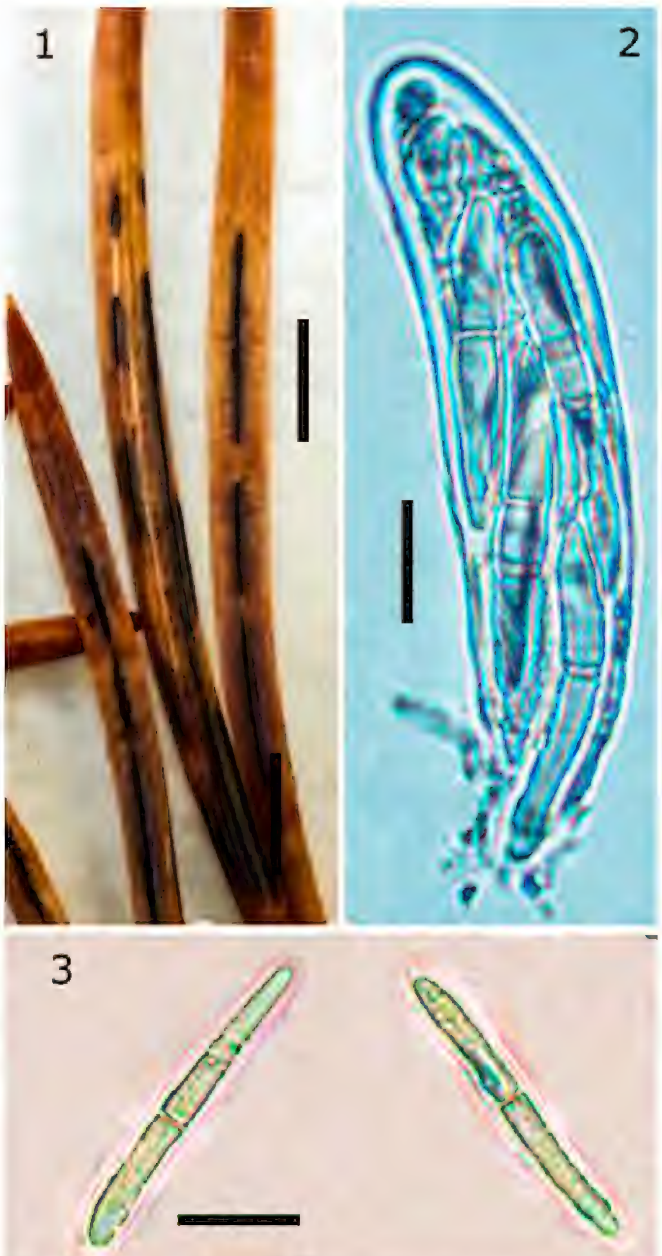
Taxonomy

Elytroderma baikalense T.I. Morozova, Lar.N. Vassilyeva & S.L. Stephenson, **sp. nov.** Figs. 1-3

MycoBank MB 817182

Differs from *Elytroderma deformans* by its smaller ascospores and the absence of witches' broom symptoms and from *E. torres-juanii* by its smaller hyaline ascospores.

Type: Russia, Irkutsk region, Lake Baikal, Pribaikalsky National Park, Olkhon Island, Olkhonsky District, 15 km east of the village of Khuzhir, on dying needles of *Pinus sylvestris*, 27 July 1998, T.I. Morozova (**Holotype,** VLA P-2934; **isotype,** IRK 45988).



Figs. 1–3. *Elytroderma baikalense* (holotype, VLA P-2934). 1. Ascomata. 2. Ascus with ascospores. 3. Ascospores. Scale bars: 1 = 3.0 mm; 2 = 20 µm; 3 = 25 µm.

Etymology: The specific epithet refers to Lake Baikal.

Ascomata embedded in brown needles or in brown portions of otherwise green needles, scattered or confluent when arranged in 2–3 rows along the needle, appearing as thin black lines, ranging from 2–3 to 15–20 mm long; forming diffuse brown zone lines across the needle; when viewed in a mid-point vertical section, the hymenium is covered by a clypeus comprising blackened fungal cells. Asci unitunicate, mostly saccate, 150–180 × 30–40 µm, with a J–negative apex, containing eight ascospores arranged in a fasciculate fashion. Ascospores clavate, hyaline, 1-septate, smooth, 60–80 × 6–8 µm, enveloped in a thin gelatinous sheath approximately 2 µm thick.

Additional specimens examined: **RUSSIA, Irkutsk region, Lake Baikal**, Pribaikalsky National Park, Olkhon Island, Tashkiney Valley, on dying needles of *Pinus sylvestris*, 19 July 1995, T.I. Morozova (IRK 45989); 15 km E of the village of Khuzhir, on dying needles of *Pinus sylvestris*, 27 August 1998, T.I. Morozova (IRK 46015); Sarayskaya Bay, on dying needles of *Pinus sylvestris*, 17 June 1997, T.I. Morozova (IRK 45990).

Notes: The two previously described *Elytroderma* species produce larger ascospores than *E. baikalense:* 90–130 × 8–10 µm for *E. deformans* and 130–165 × 9–12 µm for *E. torres-juanii. Elytroderma deformans* is known to cause a witches' broom of pine, but this has not been observed in either *E. baikalense* or *E. torres-juanii*. The ascospores of *E. torres-juanii* also differ from those of *E. baikalense* by their faintly yellowish brown coloration (Diamandis & Minter 1979).

Discussion

Needle viability in *Pinus sylvestris* is observed to be the longest on Olkhon Island for the entire region where *P. sylvestris* occurs. The island is the only place where needles remain viable on the trees for 10–12 years. Such extended viability is more characteristic of dark coniferous trees (e.g., *Abies sibirica* Ledeb. or *Picea abies* (L.) H. Karst.). The normal age of *Pinus sylvestris* needles is 4–5 years in ecologically pristine places around Lake Baikal, but this drops to only 2–3 years in the industrial centers and cities (Morozova 2004).

Three hotbeds of this newly described pine needle disease were found along the southeastern coast of Olkhon Island, with the distance between them being about 10–20 km. Over 20 years, these hotbeds did not spread but rather remained discrete. The affected trees lose as much as 75% of their foliage, and in the crown of the tree needles remain for only 1–2 years. However, the disease severity fluctuates from year to year. Sometimes, only individual needles are involved, but there are other years when all of the second-year needles become yellow and fall. The ascomata mature in August.

Although the trees in the hotbeds appear to be greatly weakened, we do not recommend cutting them. The older trees mostly have affected needles on the lower branches, which does not lead to a condition of complete blight. The young affected trees fall naturally and die out. Constant monitoring of the disease is recommended, and young plants should not be transported from Olkhon Island.

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Inocybe ahmadii sp. nov. and a new record of *I. leptocystis* from Pakistan

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Abstract—Two smooth-spored *Inocybe* species, *I. ahmadii* (a new taxon) and *I. leptocystis,* were collected from moist temperate forests of Pakistan and identified based on molecular phylogenetic analysis and assessment of macro- and micro-morphological characters. *Inocybe ahmadii* is described as new based on its smooth phaseoliform to sub-amygdaliform basidiospores, pinkish brown lamellae, white stipe surface that is brownish at the apex, broadly lageniform hyaline pleurocystidia, and similar but more frequent cheilocystidia. Our *I. leptocystis* specimen, molecularly supported as conspecific with specimens from Austria, China, Italy, and Finland, represents a first record of the species from Pakistan.

Key words—conifers, *Inocybaceae*, *Inocybe* sensu stricto, *Inocybe* subg. *Inocybe*, nrITS

Introduction

Inocybe (*Basidiomycota, Agaricales*, *Inocybaceae*) is a highly diverse genus of ectomycorrhizal fungi comprising 500–750 species, primarily in temperate areas. The number continues to increase due to new discoveries (e.g., Matheny et al. 2009, Alvarado et al. 2010, Bougher & Matheny 2011, Bougher et al. 2012, Kokkonen & Vauras, 2012, Matheny et al. 2012, Fan & Bau 2013, Braaten et al. 2014, Fan & Bau 2014, Latha et al. 2015, Esteve-Raventós et al. 2015, Jabeen et al. 2016). Twenty-eight species of *Inocybe* have been reported from Pakistan (Ahmad et al. 1997, Sultana et al. 2011, Saba et al. 2015, Jabeen et al. 2016).

The species within *Inocybe* can be recognized by their often small- to medium-sized basidiomata with spermatic, earthy, bitter almond, pelargonium, or fruit-like smell (although many species do not have any distinct smell), radially fibrous to cracked and often brownish pileus, and smooth, spinose, nodulose, or angular yellowish brown basidiospores (Solak et al. 2009). Matheny et al. (2009) subdivided *Inocybe* into seven major clades or lineages based on DNA sequence analyses and morphology.

Taxa within *Inocybe* subg. *Inocybe* [= *Inocybe* s.str.] typically have angular/nodulose or smooth spores, thick-walled pleurocystidia, a cortina, and a non- to only slightly pruinose stipe apex. Our study characterizes and identifies two species within *Inocybe* s.str. based on morphological and internal transcribed spacer (ITS) nrDNA analyses.

Materials & methods

Sampling site

Sampling was carried out during the 2014 and 2015 monsoon periods in Khanspur and Miandam of Khyber Pakhtunkhwa province, Pakistan.

Miandam is located in northeastern Swat district, a lush green valley surrounded by mountain peaks ranging from 4500 to over 6000 m a.s.l. and mostly covered with permanent snow. The region has a humid climate with average annual rainfall exceeding 1000 mm. Summers are mild: the hottest month is June, with mean 33°C maximum and 16°C minimum temperatures, and a rainfall of 120–132 mm in July–August. January, with mean –2°C minimum and 11°C maximum temperatures, is the coldest month (Sher et al. 2012).

Khanspur in Abbottabad district lies in the moist temperate western Himalayan mountain range (3000–4000 m a.s.l.; Khan 1999). The mean annual temperature is 10°C (Malik & Sukhera 2012). The area is characterized by cool summers and cold winters, with a 3°C mean winter temperature during December and January and 26°C mean summer temperature during June to September; mean annual rainfall is 1200 mm with 57% humidity (Ahmed et al. 2006).

The vegetation of both regions is dominated by coniferous trees (*Cedrus*, *Picea*, *Pinus*, *Taxus*) with deciduous trees (*Juglans*, *Populus*, *Quercus*, *Salix*) also present (Champion et al. 1965; Sheikh 1993)

Collection, isolation, and morphological study

Basidiomata were collected, tagged with their assigned sample code and collection date, and photographed. Fresh macroscopical characters were documented in detail. Colors were designated following the Munsell Soil Color Charts (1975). For subsequent anatomical and molecular analyses, specimens were dried under an electric fan heater to prevent any potential fungal attack or decomposition. Tissues were mounted in 5% KOH and spores, basidia, cystidia, pileipellis, and stipitipellis were examined under a MX4300H light microscope (Meiji Techno Co., Ltd., Japan). Line drawings

of microscopic characters were made with a Leitz camera lucida (Wetzlar, Germany). For each species, 20 basidiospores / from 1 specimen / from 1 collection (indicated as: [20/1/1]) were measured. Dimensions are given as (a–)b–c(–d), with the range b–c containing at least 90% of the measured values and extreme values (a & d) given in parentheses. Abbreviations: avl = average spore length, avw = average width, Q (quotient) = spore length/width ratio, and avQ = average quotient of all basidiospores (Liang & Yang 2011). Specimens were deposited in the Herbarium, University of the Punjab, Lahore, Pakistan (LAH).

DNA extraction, amplification & sequencing

Genomic DNA was extracted from a small piece (30–50 mg) of dried pileus and lamellae using 2% CTAB extraction buffer following the Bruns (1995) protocol with some modifications (300 µl of chloroform was combined with iso-amylalcohol (24:1), centrifuge conditions altered to 13.2 rpm for 20 min at 4°C, and the DNA pellet was resuspended in 50 µl of deionized water). The nrDNA ITS region was amplified with ITS1F as forward primer and ITS4 as reverse primer (White et al. 1990) using the Extract-N-Amp PCR kit (Sigma Aldrich, St. Louis, MO, U.S.A.). The DNA was amplified in 20 µl reaction volumes following thermocycler conditions in Gardes & Bruns (1993). Agarose gel electrophoresis was performed to visualize amplified fragments of DNA in the Uvtec gel documentation system (Avebury House, Cambridge, UK) using default settings (Sambrook & Russell 2001). PCR products were directly sequenced bi-directionally using the same primer set at Macrogen Inc., Republic of South Korea.

Molecular phylogenetic analysis

The generated sequences were assembled using BioEdit software. Nucleotide sequences were compared with the Basic Local Alignment Search Tool (BLAST) at National Center for Biotechnology Information (NCBI; U.S.A. database). Closely related sequences with identity greater than 90% and maximum query cover with 0.0 E value were retrieved from GenBank to reconstruct an ITS phylogeny. All sequences were aligned using MUSCLE (http://www.ebi.ac.uk/tools/msa/muscle). The alignment portion from CATTA to GACCT between the conserved motifs GAT to CAAA was included in analysis. MEGA6 (Tamura et al. 2013) was used for phylogenetic tree construction. A Maximum Likelihood (ML) tree of nrITS sequences was constructed using a General Time Reversible (GTR) model and nearest-neighbor-interchange (NNI) as a ML heuristic search method. 1000 bootstrap replicates were performed.

Inocybe ahmadii Farooqi, Niazi & Khalid, **sp. nov.** Figs 1, 2

MycoBank MB 817163

Differs from *Inocybe glabripes* by its lageniform pleurocystidia and numerous lageniform cheilocystidia.

Type: Pakistan, Khyber Pakhtunkhwa province, Abbottabad district, Khanspur, 3000–4000 m a.s.l., solitary on ground under *Pinus wallichiana* A.B. Jacks., 14 August 2015, Ayesha Farooqi AF14 (**Holotype**: LAH14215; GenBank KX254462).

Fig. 1. *Inocybe ahmadii* basidiome (LAH14215, holotype). Scale bars = 1 cm.

Etymology—The specific epithet refers to Sultan Ahmad, the pioneer mycologist of Pakistan, for his contributions in the field of mycology.

Basidioma small, ≤46 mm high, pale brown when dry. Pileus small, 21 mm diam, plano-convex to applanate, umbonate, umbo rather low and obtuse; dark pinkish brown (5YR1/4) at the centre, lighter towards margins, light pinkish brown (5YR4/6); surface dry, with appressed scales, weakly fibrillose towards the margin, slightly rimulose, edges appendiculate with whitish fibrils; context very thin, whitish. Lamellae adnexed, moderately crowded, alternating with lamellulae, 0.1 mm wide, buff (7.5YR9/4), turning brown when dry, edges entire to slightly fimbriate. Stipe 42 × 3 mm, central, even, surface pruinose at the apex, fibrillose towards the base, brownish at the apex, white to greyish towards the base, cortina present and fugacious. Context loosely stuffed, interior tubular. Odour not recorded.

Basidiospores [20/1/1] (7.8–)7.9–9.0(–9.2) × (4.3–)4.7–4.9(–5.0) µm, avl × avw = 8.4 × 4.7 µm, Q = (1.5–)1.6–1.8(–2.0), avQ = 1.7, ellipsoid; phaseoliform

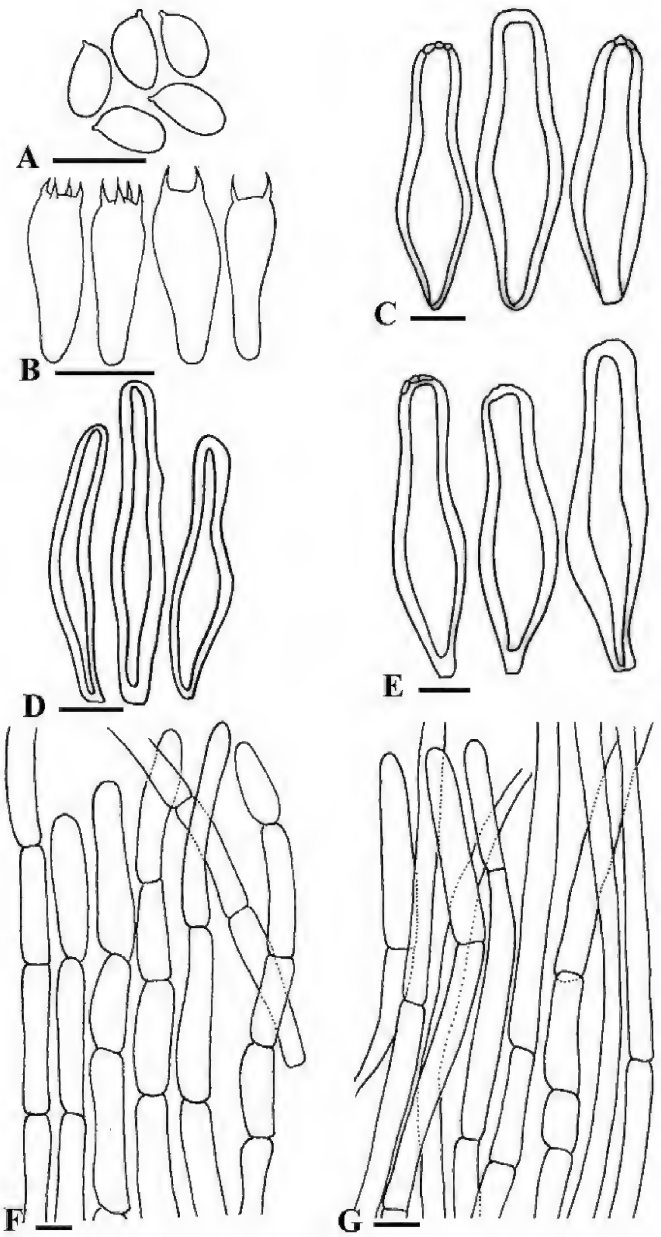

Fig. 2. *Inocybe ahmadii* (LAH14215, holotype). A. basidiospores; B. basidia; C. cheilocystidia; D. pleurocystidia; E. caulocystidia; F. hyphae from pileipellis; G. stipitipellis Scale bars: A–E = 20 µm; F,G = 40 µm. Drawings by Ayesha Farooqi.

to subamygdaliform, apiculate, mostly with obtuse apices, pale yellow brown, uniguttulate. Basidia 19.5–25 × 6.5–8.5 µm, avl × avw = 22.5 × 7.5 µm, cylindrical to clavate, 2–4-spored, hyaline. Pleurocystidia 51.5–65 × 12–17.5 µm, avl × avw = 59 × 14.5 µm, narrowly to rather broadly lageniform with obtuse apices and a small pedicel, wall ≤3 µm thick. Cheilocystidia 49–55 × 13–16.5 µm, avl × avw = 52 × 15 µm, similar to pleurocystidia but generally shorter and wider, moderately encrusted at the apex, rarely tapering at the base, hyaline, paracystidia rare. Caulocystidia slender, cylindrical, rarely subfusiform, moderately thick-walled, present only at stipe apex. Pileipellis 9–24 µm thick (av = 12.5 µm), a trichoderm, hyphae ochraceous, clamp connections present. Stipitipellis 6–14 µm thick (av = 9.0 µm), composed of cylindrical hyphae, regularly arranged, thin-walled, clamp connections present.

Inocybe leptocystis G.F. Atk., Am. J. Bot. 5: 212 (1918) Figs 3,4

Pileus 16 mm diam, yellowish brown (5YR5/8) to brown (5YR9/12) towards the margin, smooth to finely fibrillose, umbonate, margins incurved. Lamellae moderately wide, adnate, 2–4 mm wide, light brown (2.5YR2/6), edges fimbriate. Stipe 45 × 7 mm, cylindrical, brown (2.5Y3/6) to whitish (2.5YR9/12), slightly enlarged at the base; surface smooth, nowhere pruinose; central, narrower towards the apex. Context white to yellowish towards the stipe base. Odour weakly spermatic.

Basidiospores [20/1/1] (9.0–)10.1–12.5(–12.7) × (5.2–)5.4–6.4(–6.6) µm, avl × avw =11.6 × 6.0 µm, Q = (1.5–)1.7–2.2(–2.3), avQ = 1.9, smooth, subamygdaliform, yellowish brown, apiculate, sometimes slightly depressed above the apiculus, mostly uniguttulate. Basidia 28.3–35.4 × 7.1–9.2 µm, avl × avw = 33.2 × 8.6 µm, cylindrical, hyaline to yellow, 4-spored. Pleurocystidia 35–60 × 16–23 µm, avl × avw = 53 × 18 µm, with median constriction and clavate to utriform, apices obtuse or subcapitate, thin-walled, hyaline to light yellow. Cheilocystidia 52–68 × 12–24 µm, avl × avw = 62 × 17.5 µm, clavate, thin-walled, hyaline to light yellow, in various forms, rather dispersed. Caulocystidia not observed. Pileipellis 6–10.5 µm thick (av = 9.8 µm), a cutis, hyaline, composed of irregular hyphae, walls yellowish brown, clamp connections present. Stipitipellis 7–9 µm thick (av = 8.2 µm), a cutis of cylindrical hyphae, yellowish brown pigment incrusted, septate.

Material examined: **PAKISTAN, Khyber Pakhtunkhwa Province, Swat District,** Miandam at 2000 m a.s.l, under conifers, solitary, 23 Aug 2014, Sana Jabeen FA-5 (LAH35112; GenBank KX254461).

Fig. 3. *Inocybe leptocystis* basidiome (LAH35112). Scale bars = 0.5 cm.

Molecular phylogenetic results

Sequences of the *Inocybe ahmadii* PCR products using ITS1F and ITS4 were trimmed at the conserved motifs to produce consensus sequences of 643 base pairs. BLAST searches of *Inocybe ahmadii* ITS1F and ITS4 sequences revealed 93–91% identity with *Inocybe* sp. (JF908230) from Italy listed as "*Inocybe rufulovelata*", an unpublished name, in Osmundson et al. (2013) and *I. glabripes* Ricken (AJ889952) from Denmark with 94–100% query cover and 0.0 E value.

Sequencing of the *Inocybe leptocystis* PCR product using the same set of primers yielded 727–809 base pairs, which were trimmed at the conserved motifs to produce consensus sequences of 637 base pairs. The BLAST revealed a 99% similarity with *I. leptocystis* from China (KU359777) and Italy (JF908158) and a 95–94% similarity with a different species identified as *I. leptocystis* from China (KJ810596) and Sweden (AM882801). It therefore appears that the label

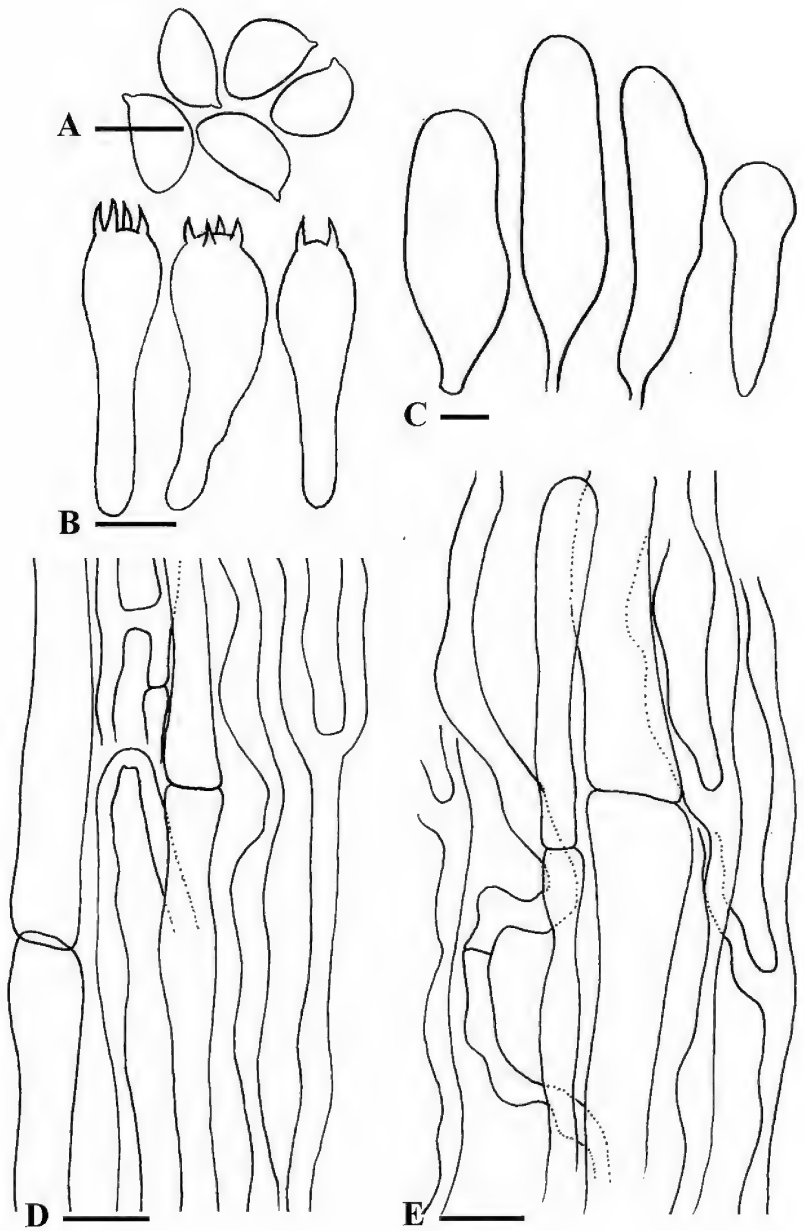

Fig. 4. *Inocybe leptocystis* (LAH35112). A. basidiospores; B. basidia; C. pleurocystidia; D. stipitipellis; E. pileipellis. Scale bars = 10 µm. Drawings by Sana Jabeen.

"*I. leptocystis*" has been applied to sequences representing at least two different species.

We compared our ITS sequences with 44 sequences retrieved from GenBank. *Inocybe dunensis* P.D. Orton (JF908262) and *I. hirculus* Vauras (AM882723) were chosen as outgroup (Fig. 5). After alignment, a 638 character long matrix was obtained, of which 352 characters were conserved, 271 variable, 194 parsimony-informative, and 76 singletons. Three major clades were formed within the ingroup sequences. *Inocybe ahmadii* clustered with *Inocybe* spp. (HG796992, JF908193) and *I. glabripes* Ricken in clade I but formed its own lineage on an isolated branch. *Inocybe leptocystis* clustered in clade II with *I. aeruginascens* Babos (labeled as "*I. bresadolae*") and *I. hystrix* (Fr.) P. Karst.

Discussion

Our analysis of sequences from *Inocybe* s.str. (clade Inocybe; Latha et al. 2016) indicated three clades I–III. Typical characters include presence of thick-walled pleurocystidia, two kinds of cheilocystidia (both pleurocystidioid cheilocystidia and clavate to sphaeropedunculate paracystidia), smooth or angular or nodulose spores with fairly large apiculi; caulocystidia and a cortina may be either present (over a variable stipe length) or absent (Kuyper 1986, as *I.* subg. *Inocybe*).

Clade I, which comprised 26 sequences split into two subgroups, is characterized by species showing a brown to pale brown or ochraceous buff colored pileus (excepting the distinctively purple brown or lilaceous *I. lanatopurpurea* Esteve-Rav. & G. Moreno), a stipe that is whitish when young then brownish, cortinate young specimens, subamygdaliform spores, and apically encrusted thick-walled pleurocystidia. Within clade I, the new species *I. ahmadii* is well separated from the other taxa, forming its own lineage with notably long branch. This phylogenetic separation is supported by its morphological differences, e.g., buff brown lamellae with pinkish tinge, a whitish stipe surface with a brownish apex, ellipsoid and phaseoliform to subamygdaliform spores, apically encrusted and narrowly to broadly lageniform pleurocystidia, and generally shorter and broader cheilocystidia. *Inocybe ahmadii* forms a subgroup with *Inocybe* sp. HG796992 (Pakistan) and JF908193 (U.S.A.) and *I. glabripes* AJ889952, AM882807 (Sweden) and JF908239 (U.S.A.), but a 86% ML bootstrap value well supports its separation from these taxa. *Inocybe glabripes* is distinguished by its olivaceous tinged ochraceous brown lamellae, its

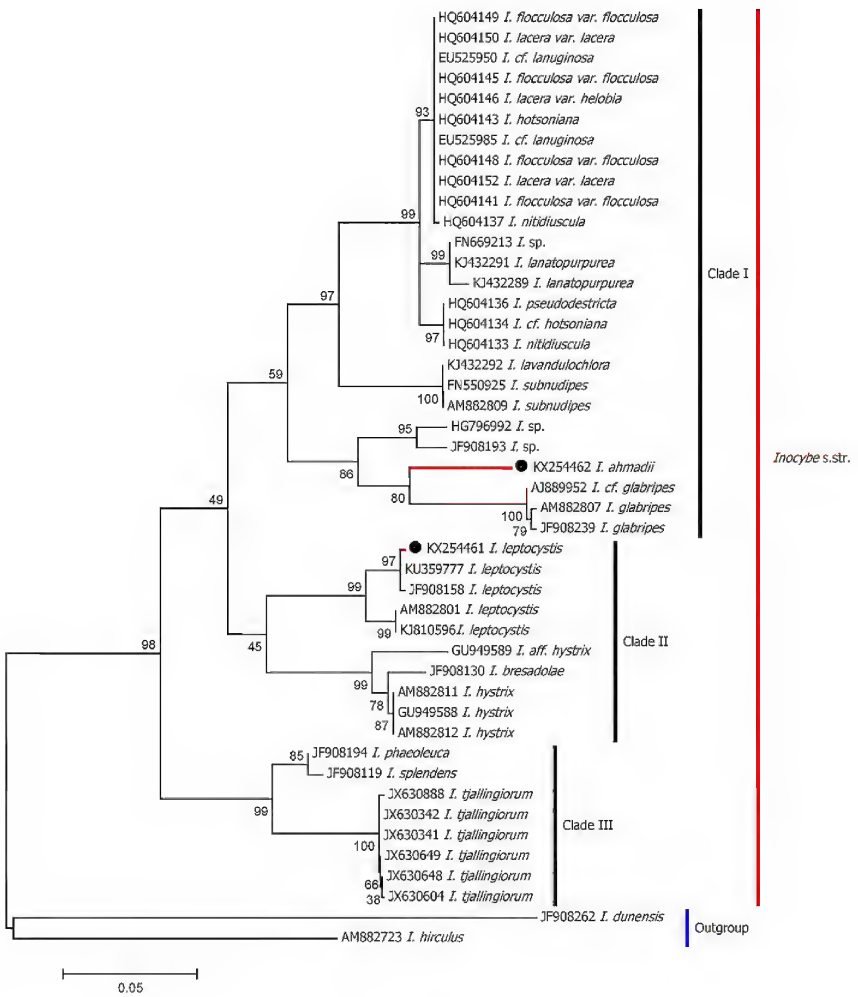

Fig. 5. The evolutionary history of species of *Inocybe* sensu stricto inferred by using the Maximum Likelihood method based on the General Time Reversible model in MEGA6. The tree with the highest log likelihood (–3363.8870) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values are indicated above or below branches. The analysis involved 46 nucleotide sequences. There were a total of 630 positions in the final dataset. The two Pakistani collections have been labeled with ● and highlighted.

ochraceous or honey brown stipe surface, subamygdaliform spores, cylindrical to subfusiform pleurocystidia, and infrequent cheilocystidia. All these morphological characters separate *I. ahmadii* from *I. glabripes*, supporting its designation as a new species.

Clade II included 10 sequences, split into two subgroups, one of which clustered our Pakistani *I. leptocystis* sequence with four other *I. leptocystis* sequences. Species of clade II can be distinguished morphologically from clade I by their lack of caulocystidia and presence of thin-walled pleurocystidia.

Inocybe leptocystis has been reported from several different temperate regions: the U.S.A. (Atkinson 1918, Kobayashi 2002), Asia (China; KU359777, Fan & Bau, unpublished), and Europe (Austria, Kobayashi 2002; Finland, Kobayashi 2002 and Matheny 2005; Italy, Osmundson et al. 2013; Sweden, Ryberg 2009).

The sequence data suggest two different taxa are labeled as "*I. leptocystis*" within clade II: group (1) from China (KU359777) and Italy (JF908158); and group (2) from Sweden (AM882801) and China (KJ810596). Morphologically, the Pakistani collection corresponds with the descriptions of the collections from Austria and China (Kobayashi 2002), and the sequence from the Pakistani collection clusters with the group (1) sequences from China and Italy. This group (1) subclade is represented by species having an ochraceous brown to buff pileus, non-pruinose smooth stipe, thin-walled, subutriform to clavate pleurocystidia (except *I. hystrix*, which has cylindrical to sublageniform pleurocystidia).

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Corynespora titarpaniensis sp. nov., on *Lepidagathis* from Central India

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Abstract—A new asexual fungus *Corynespora titarpaniensis*, discovered on *Lepidagathis* sp. (*Acanthaceae*) from Madhya Pradesh, India, is described, illustrated, and compared with closely similar species.

Key words—biodiversity, *Corynesporascaceae*, foliar diseases, phytopathogenic fungi, taxonomy

Introduction

Corynespora was described by Güssow (1906), and almost 200 species have been recorded (Index Fungorum 2016). Indian mycologists have described several novel species in the past 15 years (Dubey & Rai 2003; Jain et al. 2002; Kumar et al. 2007, 2008, 2012, 2013; Meenu & Kamal 1998; Meenu et al. 1997, 1998; Pal et al. 2007; Sharma et al. 2002a,b, 2003, 2005; Singh & Kamal 2012; Singh et al. 2000a,b, 2007a,b, 2013, 2014; Verma et al. 2008). Synoptic tables for the main morphological features of *Corynespora* spp. have been provided by McKenzie (2010) and Siboe et al. (1999).

In 2016, during routine survey for foliicolous fungi, an interesting fungus causing a serious disease was collected on living leaves of *Lepidagathis* sp. from a village in Madhya Pradesh. Based on morphological characteristics such as

colouration and septation in conidia and conidiophores, as well as mode of conidial ontogeny, this fungus was assigned as a new species of *Corynespora*. A detailed description and illustrations of the new fungus are presented here.

Materials & methods

Fresh leaf specimens with disease symptoms were collected during the course of field trips. The samples were placed in zip-lock polythene bags, taken to the laboratory, and treated according to Castañeda-Ruíz (2005). Detailed observations of morphological characters were carried out using a Leica light microscope. Specimens were prepared for microscopical observation by hand sectioning and scraping; the sections were mounted in lactophenol cotton-blue. Measurements were made of 30 conidia, hila, and conidiophores. Line drawings were prepared at a magnification of 400×. Photographs of colonies on leaves and microphotographs of fungal propagules were taken using a Sony DSC-5730 camera. The holotype is deposited in the Ajrekar Mycological Herbarium, Ajrekar Research Institute (ARI), Pune, India (AMH), and isotype material is deposited in the Mycological Herbarium of the Department of Botany of Dr. Harisingh Gour University Sagar, M.P., India (MH-DHSGU).

Corynespora titarpaniensis P. Kushwaha, Raghv. Singh & Sh. Kumar, **sp. nov.** Figs 1–4

MycoBank MB 818169

Differs from *Corynespora barleriicola* and *C. peristrophicola* by its larger, solitary conidia with more numerous septa.

Type: India, Madhya Pradesh, Sagar, Deori, Titarpani village, 23.3898°N 79.0165°E, on living leaves of *Lepidagathis* sp. (*Acanthaceae*), Jan. 2016, coll. Prakash Kushwaha (**Holotype,** AMH 9786; **isotype,** MH-DHSGU 188).

Etymology: Latin, *titarpaniensis*, referring to the type locality.

Infection spots amphigenous, scattered, initially circular to subcircular, 1–9 mm in diam., later becoming irregular and spreading over entire leaf surface, more or less necrotic, brown to blackish brown. Colonies amphiphyllous, effuse, brown to blackish brown. Mycelium internal, thin-walled, branched, smooth, brown to blackish brown. Stromata absent or rarely present. Conidiophores macronematous, arising singly or rarely in groups of 2–7, cylindrical, erect to procumbent, straight to flexuous, smooth, unbranched, thick-walled, 5–16-septate, constricted at septa, medium brown to blackish brown, 155–500 × 5–11.5 µm, with numerous successive cylindrical enteroblastic percurrent extensions, basal cell swollen. Conidiogenous cells monotretic, integrated, terminal, scars unthickened. Conidia solitary, smooth, simple, thin-walled, straight to slightly curved or wavy in margin, obclavate-cylindrical, apex obtuse to

Fig. 1. *Corynespora titarpaniensis* on *Lepidagathis* sp. a, b. Infected host plant. c, d. Infection spots on upper surface. e, f. Infection spots on lower surface. Scale bars = 10 mm.

rounded, 5–35-distoseptate with some transverse band-like eusepta, with some deep constrictions, 50–340 × 5–20 µm, olivaceous to light brown, hilum rounded to obconico-truncate, thickened, 3–5 µm diam.

Discussion—Two other *Corynespora* species have been described from acanthaceous hosts: *C. barleriicola* N. Sharma et al. and *C. peristrophicola* Raghv. Singh & Kamal. The conidia of *C. barleriicola* (41–246 × 10–18.5 µm,

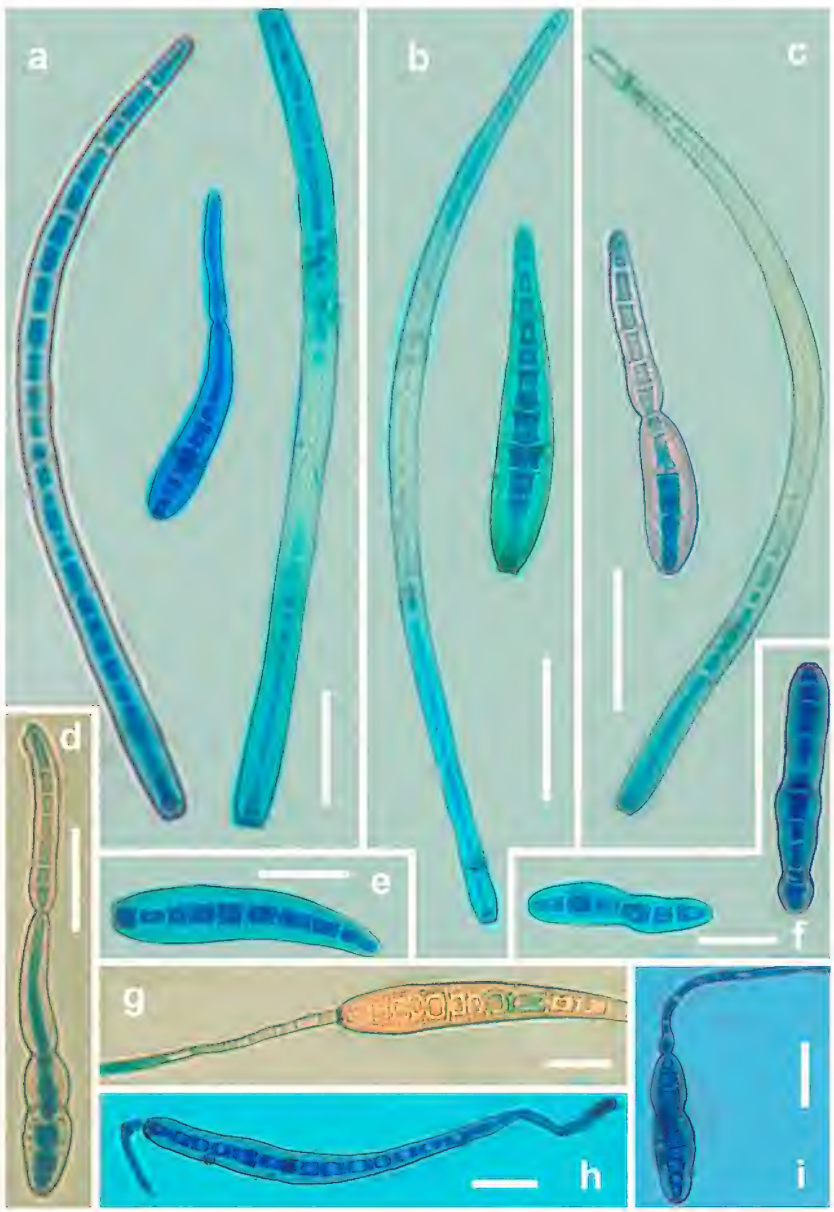

Fig. 2. *Corynespora titarpaniensis* (holotype, AMH 9786). a–f. Conidia. g–i. Germinating conidia. Scale bars: a–c = 50 µm; d–i = 20 µm.

FIG. 3. *Corynespora titarpaniensis* (holotype, AMH 9786). a. Solitary conidiophore. b. Conidiophores in fascicle. c–e. Bulbous basal cell of conidiophores. f–h. Developing conidia at conidiogenous loci. Scale bars: a, b = 50 µm; c, d = 20 µm; e–h = 40 µm.

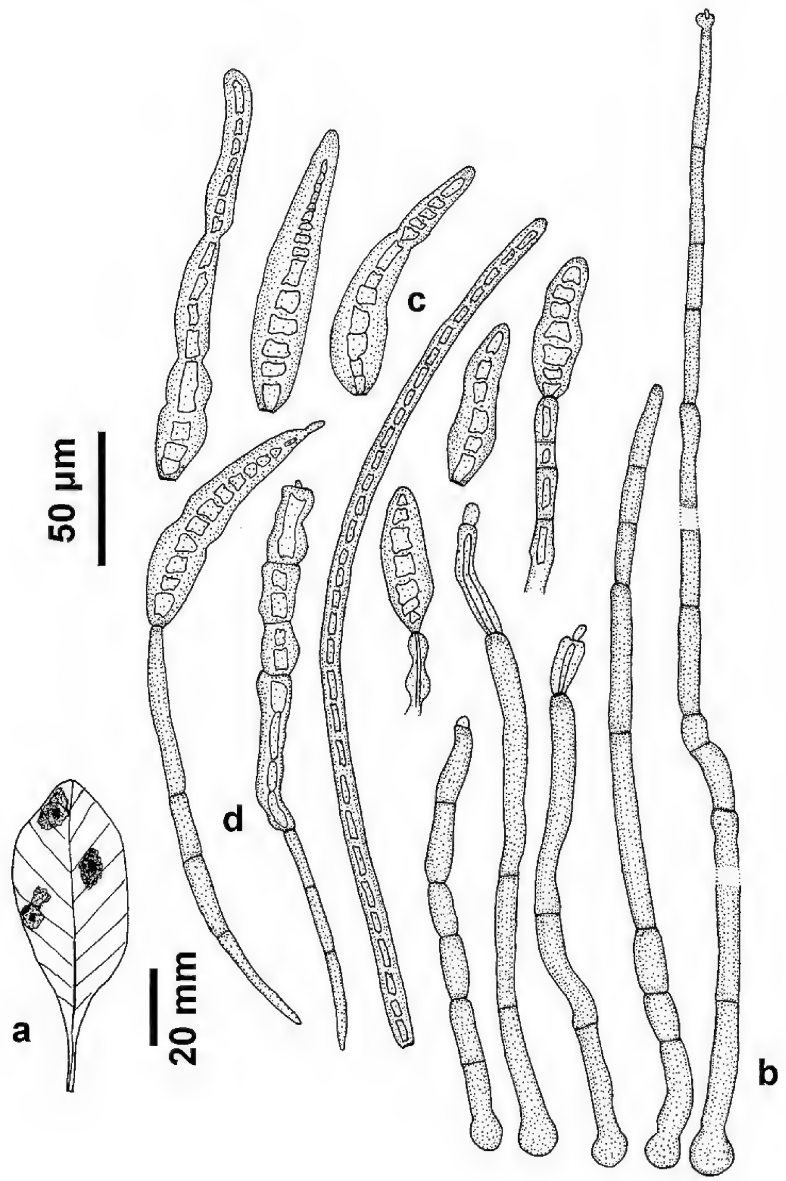

Fig. 4. *Corynespora titarpaniensis* (holotype, AMH 9786).
a. Infection spots. b. Conidiophores. c. Conidia. d. Germinating conidia.

3–14-septate; Sharma et al. 2002b) and *C. peristrophicola* (60–135 × 5–16 µm, 5–12-septate; Singh & Kamal 2012) are catenate and shorter and have fewer septa than those of *C. titarpaniensis*.

The conidia of *C. azadirachtiana* N. Sharma et al. (32–303.5 × 7–21.5 µm, 1–20-septate; Sharma et al. 2002b), *C. colebrookiana* N. Sharma et al. (45–330 × 6–22 µm, 4–16-septate; Sharma et al. 2002b), *C. catharanthicola* Z.D. Jiang & P.K. Chi (140–310 × 5.5–11 µm, 4–25-septate; Chi 1994) and *C. proliferata* Loer. (30–300 × 9–12 µm, 3–17-septate; Loerakker 1975) are catenate and have fewer septa than *C. titarpaniensis*.

Corynespora leucaenae N. Sharma et al. (16–298 × 10–19 µm, 1–28-septate; Sharma et al. 2003), *C. trematicola* N. Sharma et al. (104–296 × 11–16 µm, 1–12-septate; Sharma et al. 2002a, as "*tremicola*") and *C. longispora* A.K. Sarbhoy & Saikia (120–330 × 5.5–8 µm, 11–24-septate; Saikia & Sarbhoy 1981) also have conidia similar to those of *C. titarpaniensis* but differ by having fewer septa.

The conidia of *C. smithii* (Berk. & Broome) M.B. Ellis (70–410 × 12–19 µm, 7–45-septate; Ellis 1957) and *C. viticus* Y.L. Guo (80–383 × 6–9 µm, multiseptate; Guo 1984) appear close to *C. titarpaniensis* but differ by their catenate, cylindrical, longer, thick-walled conidia with more septa.

The new species also resembles *C. rhododendri* K. Zhang & X.G. Zhang, which differs by its much longer, thinner, obclavate to long rostrate conidia (180–400 × 7.5–11 µm, 19–36-septate; Zhang et al. 2008).

Another similar species, *C. micheliae* Z.Q. Shang & X.G. Zhang differs from *C. titarpaniensis* by its rostrate conidia (333–360 × 15–19 µm, 12–28-septate) with its upper cells gradually tapering to a narrow apex, a wider hilum (6–7 µm), and a darker brown lower cell; and by its shorter, wider conidiophores (190–210 × 9–19 µm; Shang & Zhang 2007).

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Morganella austromontana sp. nov. from the South Brazilian Plateau

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Abstract—A new *Morganella* species was collected during field exploration of the mycobiota of the South Brazilian Plateau. It is proposed here as *Morganella austromontana*, distinguished by its ovoid basidiospores and its soil habitat. A complete description and illustrations of its morphological features are accompanied by a taxonomic discussion.

Key words—*Agaricaceae*, *Agaricomycetidae*, *Araucaria angustifolia* forest, puffballs, taxonomy

Introduction

Morganella Zeller is diagnosed by small gasteroid basidiomata (puffballs) that are globose, subglobose or depressed enclosed within a double peridium comprising a granular, verrucose, velutinous or spiny exoperidium and an endoperidium with an apical pore; basidiospores are globose or subglobose (rarely ovoid), a eucapillitium is absent, and a paracapillitium is present (Zeller 1948, Kreisel & Dring 1967). About 20 species have been included in the genus. From Brazil, 12 species have been reported and/or described in the last decade (Cortez et al. 2007; Trierveiler-Pereira & Baseia 2009; Barbosa et al. 2011; Alfredo et al. 2012, 2014; Alfredo & Baseia 2014; Alves & Cortez 2013). These puffballs occur in diverse Brazilian biome habitats, including arid environments (Barbosa et al. 2011) and seasonal ombrophilous forests (Alfredo et al. 2012, Alves & Cortez 2013) where they grow on rotting wood among litter and mosses.

During a survey of the gasteroid mycobiota in South Brazilian forests in the Atlantic Forest biome, a new *Morganella* species was found growing in the mixed ombrophilous forest typical of south Brazil. Here we describe this species as *Morganella austromontana.*

Materials & methods

Specimens were gathered during field trips in the natural reserves of the Rio das Furnas in the municipality of Alfredo Wagner (27°42′00″S 49°20′02″W), State of Santa Catarina, and the National Park of Aparados da Serra in the municipality of Cambará do Sul (29°08′02″S 49°59′40″W), State of Rio Grande do Sul. Both sites are covered by *Araucaria angustifolia* forests and are located on the South Brazilian Plateau at altitudes of 900–1200 m (Veloso et al. 1991). Köppen (1948) classified the region's climate as Cfb: mesothermic, wet (with regular rainfall throughout the year), and with mild summers.

The specimens were examined morphologically according to Miller & Miller (1988). Colors were coded according to the color chart of Kornerup & Wanscher (1978). Measurements and photographs were taken using a Motic BA310 microscope equipped with a Moticam 2500 digital camera and Motic Image Plus 2.0 software. Scanning electron microscopy (SEM) was performed at the Center of Electronic Microscopy of the Universidade Federal do Rio Grande do Sul (CME/UFRGS) under a Jeol JSM-6060 SEM. All studied specimens are preserved at the Herbarium, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (ICN). Five collections from Rio Grande do Sul conserved in the ICN Herbarium representing species closest to *M. austromontana* were also examined.

Taxonomy

Morganella austromontana C.R. Alves, Cortez & R.M. Silveira, **sp. nov.** Figs 1–6

MycoBank MB 820972

Differs from *Morganella subincarnata* by its lighter exoperidium, its ovoid basidiospores, and its habitat on soil in araucariaceous forest.

Type—Brazil, Santa Catarina State: Alfredo Wagner, Reserva do Rio das Furnas, 7.I.2014, leg. C.R. Alves 94 (**Holotype**, ICN 181431).

Etymology—The name, meaning "from the southern mountains," refers to the montane type region on the South Brazilian Plateau.

Basidiomes 10 mm high, 8–20 mm diam., subglobose to pyriform. Exoperidium spiny, formed of short spines (<1 mm high), numerous and densely grouped on the top of the basidioma, light brown (6D5) when fresh, falling away as plates detach from the endoperidium when mature. Endoperidium papery, smooth to areolate, white (1A1) when fresh, then yellow grey (2B2) after dried. Sterile Base poorly developed to present, reaching 3 mm high, yellowish grey (2B2), with scattered, white and thin rhizomorphs (<1 mm thickness).

Figs 1–3. *Morganella austromontana* (holotype ICN 181431). 1. Basidiome in situ. 2. Dried basidiomata. 3. Paracapillitium and basidiospores. Scale bars: 1, 2 = 10 mm; 3 = 10 µm.

Gleba cottony, brownish grey (2D2). Subgleba present, ≤3 mm high, cellular, greyish yellow (1B3). Basidiospores 4–5 × 3–4 µm, ovoid to subglobose, pale green in 5% KOH, surface verrucose under light microscope, spiny under SEM;

spines cone-shaped, <2 µm high. Paracapillitium 7–10 µm diam., inflated, with numerous small pores c. 1 µm diam., amorphous matter surrounding the hyphae, septa regular, sometimes, with dichotomous tips. Exoperidium hyphae 10–26 × 8–15 µm diam., globose, subglobose or pyriform, hyaline to pale yellow, walls thin, about 1 µm. Mycosclereids 25–80 × 7–13 µm diam., irregular in shape, hyaline, walls thick (3 µm) and smooth.

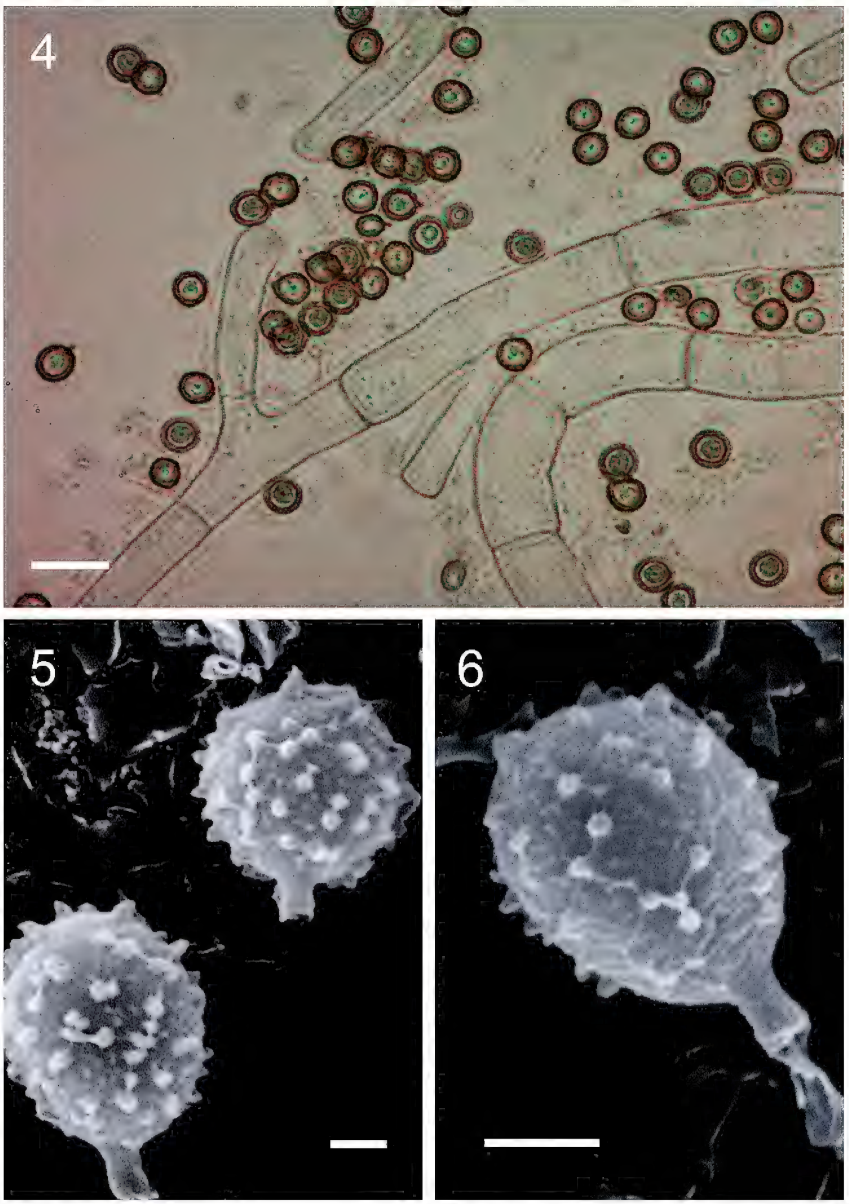
Ecology & distribution—Basidiomata solitary to gregarious, growing on soil, among a few herbs on the trail board in the Mixed Ombrophilous Forest, with *Araucaria angustifolia* (Bertol.) Kuntze (*Araucariaceae*; Paraná pine, Brazilian pine), about 480–900 m altitude, during rainy summer. Only known from the South Brazilian Plateau (States of Rio Grande do Sul and Santa Catarina).

Additional specimens examined — **BRAZIL. Rio Grande do Sul State: Cambará do Sul, Parque Nacional dos Aparados da Serra,** 13.III.2014, leg. A. Gerlach 1486 (ICN 181432); **São Francisco de Paula,** Veraneio Hampel, 8.I.2006, leg. V.G. Cortez 013/06 (ICN 154553); FLONA, 21.IV.2007, leg. V.G. Cortez 048/07 (ICN 154554); **Itaara,** Parque Pinhal, 4.V.2007, leg. V.G. Cortez 075/07 (ICN 154555); **Santa Maria**, FEPAGRO, 15.V.2007, leg. V.G. Cortez s.n (ICN 154556); Campus UFSM, 4.VI.2007, leg. G. Coelho 485-7 (ICN 154557).

Comments—*Morganella austromontana* is described as a new based on its combination of mainly ovoid ornamented basidiospores (verrucose under LM; shortly spiny under SEM) and an exoperidium densely covered with short white spines in younger basidiomata that become pale brownish and fall as plates in maturity. Another diagnostic feature is the relatively wide (≤10 µm) diameter of paracapillitium hyphae, which is rarely greater than 5–6 µm in other species. The thick-walled mycosclereids found in the exoperidium of *M. austromontana* have also been observed in *M. sulcatostoma* C.R. Alves & Cortez (Alves & Cortez 2013).

Morganella austromontana can be nested among members of *Morganella* sect. *Subincarnata* sensu Ponce de Leon (1971), a section that accommodates species with a deciduous exoperidium and reticulate-areolate endoperidium. Within *M.* sect. *Subincarnata*, *M. costaricensis* M.I. Morales, which shares with *M. austromontana* an exoperidium with deciduous spines and areolate endoperidium, can be separated by its smooth globose to subglobose basidiospores (Morales et al. 1974, Suárez & Wright 1996).

The type species of the section, *Morganella subincarnata* (Peck) Kreisel & Dring, appears related to *M. austromontana* based on its similar pattern of exo- and endoperidium but differs in its darker (olivaceous brown) exoperidium, its globose basidiospores (3.5 × 6.5 µm diam.), and its preferred substrate on



Figs 4–6. *Morganella austromontana* (holotype ICN 181431). 4. Basidiospores and paracapillitium. 5, 6. Basidiospores (SEM). Scale bars 4 = 10 µm; 5, 6 = 1 µm.

angiospermous wood (Bowerman 1961, as *Lycoperdon subincarnatum*; Coker & Couch 1928, as *Lycoperdon subincarnatum*; Kreisel & Dring 1967; Ponce de Leon 1971).

Another noteworthy feature of *Morganella austromontana* is the fact its basidiomata were collected from soil, instead of rotting wood, the usual substrate for most *Morganella* species. However, a soil substrate is not exclusive to *M. austromontana;* for example, *M. arenicola* Alfredo & Baseia grows on sandy soil (Alfredo et al. 2014).

Further fieldwork will be necessary to determine if the proposed species is, in fact, restricted to the southern Brazilian highlands and, in particular, to *A. angustifolia* forests. However, based on the current available data and ongoing survey of the gasteroid fungi of Southern Brazil, *M. austromontana* appears strongly associated with these environments.

Acknowledgments

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Microidium phyllanthi-reticulati sp. nov. on *Phyllanthus reticulatus*

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Abstract—A new powdery mildew species of the genus *Microidium* (*Erysiphaceae*) found on *Phyllanthus reticulatus* (*Phyllanthaceae*) in Thailand is described. *Microidium phyllanthi-reticulati* [= *Oidium phyllanthi* var. *reticulati*] is characterized by having amphigenous colonies on the host surface, long conidiophore foot-cells twisted at the base, and barrel-shaped to cylindrical conidia with *Microidium*-type germ tubes. The new species differs from the allied and morphologically very similar *M. phyllanthi* in producing smaller conidia and conidiophores with longer foot-cells. Analyses of rDNA ITS + 28S sequence data support the separation of *M. phyllanthi-reticulati* from *M. phyllanthi*. The new species is described and illustrated in detail, and compared with other species assigned to *Microidium*.

Key words—Asia, *Erysiphales*, phylogeny, *Euphorbiaceae*

Introduction

The name *Oidium phyllanthi* J.M. Yen was originally proposed for a powdery mildew found on *Phyllanthus urinaria* in Taiwan; two types of conidia and conidiophores were described—primary conidiophores with cylindrical to oval conidia and secondary conidiophores producing with cylindrical conidia (Yen 1967). Subsequently *O. phyllanthi* has also been recorded on *Phyllanthus acidus*, *P. amarus*, *P. niruri*, *P. reticulatus*, and *P. rheedei* (Braun & Cook 2012, as *Microidium phyllanthi*). Narayanswami & Ramakrishnan (1969) published an illegitimate later homonym, *O. phyllanthi* Narayanas. & K. Ramakr., based on an Indian collection on *Phyllanthus niruri* var. *amarus* [≡ *P. amarus*], but provided only a meagre description confined to the size of conidia (14.5–20

× 2.2–9.1 µm); Hosagoudar (1991) introduced *O. ramakrishnanii* Hosag. as a legitimate replacement name for this species.

Oidium subg. *Microidium* To-anun & S. Takam. was introduced by To-anun et al. (2005) based on molecular phylogenetic analyses of the rDNA of 18S and 28S regions and a morphological re-assessment of specimens of *Oidium phyllanthi* found on *Phyllanthus acidus*, *P. amarus*, and *P. reticulatus*. The specimens concerned were unique due to the formation of a different type of conidial germination, designated as *Microidium*-type (To-anun et al. 2002). Subsequent molecular analysis using a 18S and 28S rDNA (D1 and D2 regions) combined dataset indicated that the sequences from *O. phyllanthi* on *P. acidus*, *P. amarus*, and *P. reticulatus* represented a unique lineage within the *Erysiphaceae* and that the sequence of a *P. reticulatus* isolate differed from the *P. acidus* and *P. amarus* isolates by 1.38% in the 28S rDNA D1/D2 sequence (To-anun et al. 2005).

Braun & Cook (2012: 624) later raised *Oidium* subg. *Microidium* to generic rank as *Microidium* (To-anun & S. Takam.) To-anun & S. Takam. Here we present ITS + 28S analyses of *Microidium* on *Phyllanthus*, including new sequences from 15 specimens and additional sequences retrieved from GenBank, together with detailed morphological examinations of all of these specimens.

Materials & methods

Morphological examination

Specimens were collected in northern Thailand between November 2004 and January 2016. The host name, collection data, localities, and the name of the collector were noted. Morphological examinations were carried out as outlined in Meeboon & Takamatsu (2015a). Hyphae, conidiophores, and conidia were stripped from the leaf surfaces with a clear adhesive tape, mounted on a microscope slide with the fungal mycelium upper most, and examined in water using a light microscopy with phase contrast at 20×, 40×, and 100× (oil immersion). Thirty conidia were measured from each specimen examined.

Conidial germ tubes were examined using the method of Hirata (1942). The inner surface cell layer of onion scales was cut with a razor in a size of 1 cm^2 and stripped off by a clean forceps. The cell layer was kept in 80% ethanol more than two weeks and rinsed with tap water for 30 min before use. The cell layer was put on a microscope slide, followed by removing excess water with filter paper, and powdered with the conidia. The conidia-covered cell layer, floating on distilled water in a Petri dish, was incubated at 20–25°C for 24h until microscopic examination.

Specimens were deposited in the herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS), and Mie University Mycological Herbarium, Tsu, Japan (MUMH).

Phylogenetic analysis

Whole-cell DNA was extracted by the Chelex method (Walsh et al. 1991, Hirata & Takamatsu 1996). The nucleotide sequences of the 5′-end of the 28S rRNA gene (including domains D1 and D2) were determined in accordance with Meeboon & Takamatsu (2015b). The rDNA internal transcribed spacer (ITS) region including 5.8S rDNA was amplified using primers ITS5 (White et al. 1990) and p3 (Kusaba & Tsuge 1995) for the first amplification. One microlitre (μl) of the first reaction mixture was used for the second amplification with the partial nested primer sets ITS5 and ITS4 (White et al. 1990). The ITS5/ITS4 fragment was subjected to sequencing using ITS1, ITS4, T3, and T4 (Hirata & Takamatsu 1996). Representative sequences gained in this study were deposited in DNA databases (DDBJ, EMBL, GenBank). These sequences were aligned with the 28S rDNA D1/D2 sequence in To-anun et al. 2005 using MUSCLE (Edgar 2004) implemented in MEGA version 6 (Tamura et al. 2013). Alignments were further manually refined using MEGA and deposited in TreeBASE (http://www.treebase.org/) under the accession number S12714. Phylogenetic trees were obtained from the datasets by using the maximum parsimony (MP) method implemented in PAUP* 4.0b10 (Swofford 2002) with heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses (Felsenstein 1985) using 1000 replications with step-wise addition option set as simple. BS values 70% or higher were given. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

Taxonomy

Microidium phyllanthi-reticulati Meeboon & S. Takam., **sp. nov.** FIG. 1

MYCOBANK MB 800332

= *Oidium phyllanthi* var. *reticulati* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal, Indian Phytopathol. 57: 479. 2004 [as "*reticulatus*"].

Differs from *Microidium phyllanthi* by its smaller conidia and its longer foot-cells.

TYPE—Thailand, Chiang Mai province, Chiang Mai University, on living leaves of *Phyllanthus reticulatus* Poir. (*Phyllanthaceae*), 22 December 2004, J. Meeboon (**Holotype**, TNS-F-46801; **isotype**, MUMH 3364; GenBank AB719952, LC259489).

ETYMOLOGY—Named after the host species, *Phyllanthus reticulatus*.

COLONIES amphigenous, mycelium white, effuse, confluent, forming irregular white patches or covering the whole leaf surface (also on young stems); hyphae hyaline, branched, septate, 3–5 μm diam., thin-walled, smooth. APPRESSORIA well developed, lobed to multilobed, opposite in pairs or single. CONIDIOPHORES formed singly on hyphal cells, arising ± centrally from the upper cell surface, 82–183 × 4–8 μm, foot-cells long, cylindrical, twisted at the base, 40–118 ×

4–8 µm, with a basal septum near the junction with the supporting hypha or elevated up to 5 µm, conidiophores producing 3–6 conidia in chains (catenescent). Conidia cylindrical to doliiform cylindrical, (7–)15–20(–23) × (7–)8–10(–13) µm, with a large length/width ratio of 1.8–2.5, containing oil-drop-like inclusions but devoid of conspicuous fibrosin bodies, producing perihilar germ tubes ("on shoulders"), broadly club-shaped, terminating in nipple-shaped or lobed appressoria (*Microidium*-type).

Additional specimens examined—**THAILAND**, **Chiang Mai**, 27 December 2002, J. Meeboon (MUMH 3363; GenBank AB719953); 5 January 2007, J. Meeboon (MUMH 3824; GenBank AB719954). **Chiang Rai**, 12 January 2008, J. Meeboon (MUMH 3823; GenBank AB719955); **Nan**, 3 February 2002, S. Kom-un (MUMH 1761; GenBank LC259486, AB120758).

Comparative specimens examined—***Microidium phyllanthi***: **THAILAND**, **Chiang Rai**, 8 March 2004, J. Meeboon (MUMH 3361; GenBank AB719943); 8 March 2004, J. Meeboon (MUMH 3360; GenBank AB719944); 2 November 2013, J. Meeboon (MUMH 5743; GenBank LC259492); 18 December 2015, J. Meeboon (MUMH 6624); 26 December 2016, J. Meeboon (MUMH 6880, GenBank LC259488); 3 January 2016, J. Meeboon (MUMH 6668; GenBank LC169073, LC259490); 15 December 2014, J. Meeboon (MUMH 6676; GenBank LC169074, LC259491); 3 November 2013, J. Meeboon (MUMH 5744; GenBank LC259493). **Mae Hong Son**, 26 November 2003, J. Meeboon (MUMH 3366; GenBank AB719945). **Chiang Mai**, 13 February 2002, J. Meeboon (MUMH 1492; GenBank AB719946); 13 February 2002, J. Meeboon (MUMH 3362; GenBank AB719947); 25 February 2004, J. Meeboon (MUMH 3357; GenBank AB719948); 25 February 2004, J. Meeboon (MUMH 3822; GenBank AB719949); 3 February 2007, J. Meeboon (MUMH 3352; GenBank AB719950); 3 February 2007, J. Meeboon (MUMH 1782; GenBank LC259487, AB120755). **Nan**, 3 February 2002, S. Kom-un (MUMH 1778; GenBank AB719951, AB120754).

Phylogenetic analysis

The combination of ITS + 28S sequences from 20 specimens of *Microidium* from *Phyllanthus* (including the three specimens included in the analyses of To-anun et al. 2005: MUMH 1761, 1778, and 1782) were aligned and analysed with *Neoerysiphe galeopsidis* (DC.) U. Braun (MUMH 630) as outgroup. The alignments were carefully edited manually, with ambiguously aligned sites deleted from further analysis. Of the remaining 1239 total characters, 1036 were constant, 108 were variable but parsimony uninformative, and 95 were parsimony-informative. The MP analysis produced about 200K equally parsimonious trees with 211 steps. Topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length; a typical tree is shown in Fig. 2. The sequences obtained from the 15 specimens of *M. phyllanthi* from *P. acidus* and *P. amarus* were identical. The sequences from the five specimens from *P. reticulatus* formed a separate clade

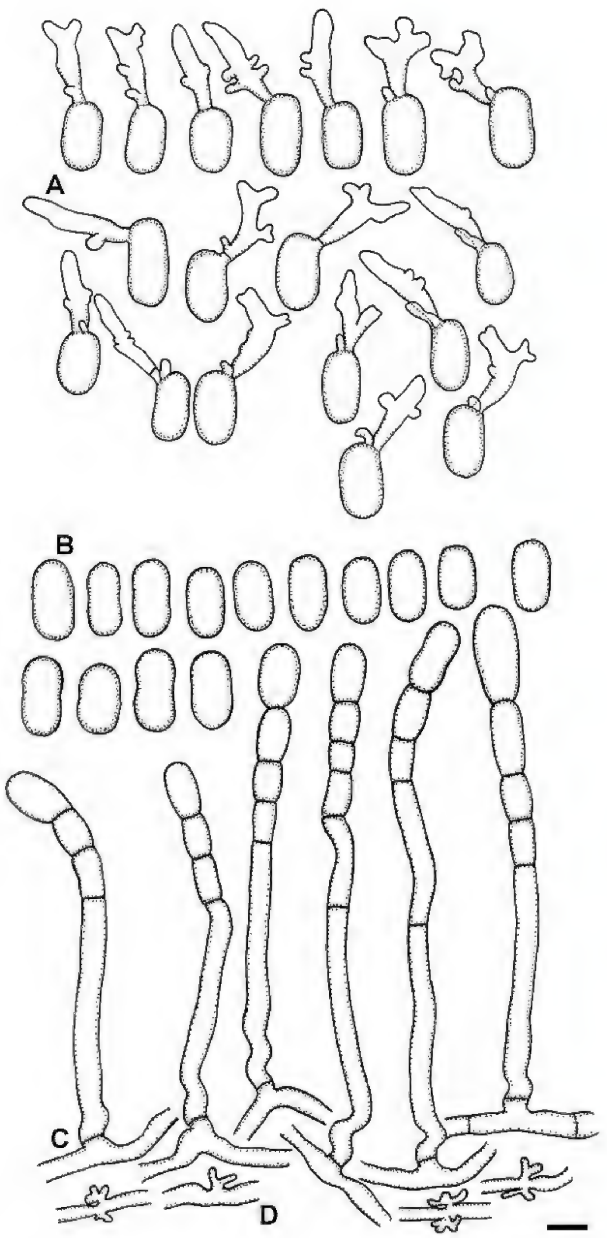

Fig. 1. *Microidium phyllanthi-reticulati* on *Phyllanthus reticulatus* (holotype, TNS-F-46801): A. Conidia with germ tubes; B. Conidia; C. Conidiophores; D. Appressoria. Scale bar = 10 µm.

with 100% bootstrap support; they differed from the sequences from the *P. acidus* and *P. amarus* specimens by 13.2% (ITS) and 4.42% (28S).

Discussion

Braun & Cook (2012) recognized three species currently assigned to *Microidium*, *M. phyllanthi* (J.M. Yen) To-anun & S. Takam., *M. bauhiniicola* (U. Braun & Dianese) U. Braun & Dianese, and *M. agatidis* (É.E. Foëx) U. Braun. *Microidium phyllanthi* is distinguished from *M. agatidis* and *M. bauhiniicola* by conidiophores with foot-cells that are twisted at the base (Braun & Cook 2012). The foot-cells of *M. agatidis* and *M. bauhiniicola* are straight, and both species are separated based on the sizes of their foot-cells, hosts, and differences in the geographical distribution: *M. agatidis*, with 15–35 µm long foot-cells, was found on *Sesbania grandiflora* in Asia, whereas *M. bauhiniicola*, with 30–90 µm long foot-cells, was found on *Bauhinia* spp. in South America (Braun & Cook 2012).

Ahmad et al. (2004) described a new variety, *O. phyllanthi* var. *reticulati*, based on the size of the conidia—slightly smaller than in *O. phyllanthi* J.M. Yen [≡ *Microidium phyllanthi*] and slightly larger than in the illegitimate *O. phyllanthi* Narayanas. & K. Ramakr. [≡ *Oidium ramakrishnanii*]. Ahmad et al. (2004) also emphasized that these three entities should be considered different varieties of *O. phyllanthi*. The relation between *Microidium phyllanthi* s. str., described from Taiwan on *P. urinaria*, and collections on *P. acidus* and *P. amarus* (available name: *Oidium ramakrishnanii*) currently cannot be clarified due to lack of sequence data from Taiwanese or other *Microidium* collections on *P. urinaria*.

The powdery mildew specimens found on *P. reticulatus* in Thailand are morphologically very similar to the original description of *O. phyllanthi* var. *reticulati* [conidia (7–)15–20(–23) × (7–)8–10(–13) µm vs. 12.7–26.7 × 6–8 µm in *O. phyllanthi* var. *reticulati*] found on the same host species. The colonies in the specimens found in Thailand grow on both sides of the leaf surface (amphigenous), whereas in *O. phyllanthi* var. *reticulati* they were described as often formed epiphyllously. Ahmad et al. (2004) did not describe the conidial germination.

Compared to the *Microidium* species keyed out in Braun & Cook (2012), the new species is morphologically close to *M. phyllanthi* based on having conidiophore foot-cells twisted at the base. However, *M. phyllanthi-reticulati* differs from *M. phyllanthi*, which has larger conidia (20–30(–40) × 7–11.5 µm) and shorter foot-cells 20–105 µm (Braun & Cook 2012). *Microidium phyllanthi*

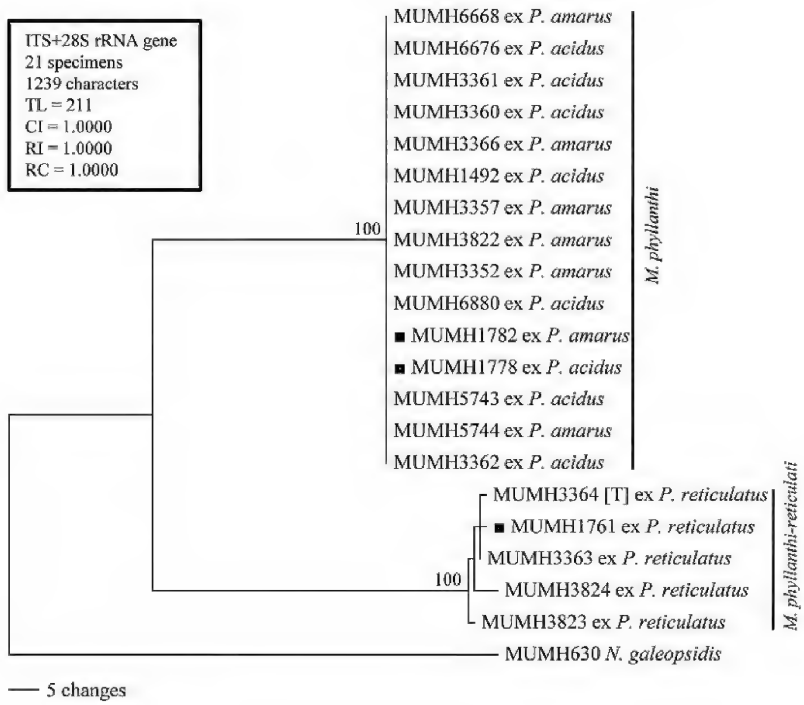

FIG. 2. Phylogenetic analysis of the nucleotide sequences of ITS + 28S regions for 21 specimens of *Microidium* from *Phyllanthus*; the three specimens included in the analyses of To-anun et al. (2005) are marked with '■'. The isotype specimen of *M. phyllanthi-reticulati* is indicated with '[T]'. The tree is one of 200K equally parsimonious trees with 211 steps, which was obtained by the parsimony method. Gaps were treated as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. Percentage bootstrap support ≥50% (1000 replications) is shown on branches.

is widespread in Africa (Ghana, Mauritius) and Asia (India, Indonesia, Sri Lanka, Taiwan, Thailand), whereas *M. phyllanthi-reticulati* is known only from India and Thailand. Furthermore, the phylogenetic analyses of the ITS + 28S regions of the *Phyllanthus* powdery mildews examined support segregation of *M. phyllanthi-reticulati* on *P. reticulatus* from *M. phyllanthi* on *P. acidus* and *P. amarus* (FIG. 2).

We refrain from using the name *O. phyllanthi* var. *reticulati* as basionym for a new combination at species level since neither the varietal type material nor any other Indian specimens were available for morphological and molecular examinations. Additionally, the protologue cited an incorrect orthographic

variant of the varietal epithet ("*reticulatus*"), and other incorrect variants ("*reticulatum*" and "*reticulatae*") have been listed in databases and in the literature; the correct epithet orthography is "*reticulati*" (Latin genitive case of the host epithet "*reticulatus*"). Nomenclaturally, our introduction of a new species name is legitimate, since varietal names do not have priority at species level (McNeill et al. 2012; Art. 11.2).

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Xylaria nelumboniformis sp. nov. from tropical China

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Abstract—A new pyrenomycetous fungus from the tropical forest of Hainan Province, southern China, *Xylaria nelumboniformis,* is described and illustrated. The new species is characterized by having lotus-leaf shaped fertile stromatal apices, a flattened surface with conspicuously tomentose edges, and navicular to crescent-shaped ascospores (23–27.5 × 7.5–9 µm) with a short oblique germ slit.

Key words—*Ascomycota,* taxonomy, *Xylariaceae*

Introduction

Wood-rotting fungi are an important component of forest ecosystems, where they decompose forest litter and maintain energy flow and material circulation (Lonsdale et al. 2008). Most *Xylaria* species produce stromata on decayed wood (Dennis 1956, Martin 1970, Rogers et al. 1987, Læssøe 1987, Van der Gucht 1995, Ju & Rogers 1999, San Martín et al. 2001, Ju et al. 2009, Hsieh et al. 2010, Fournier et al. 2011, Fournier 2014), and they are critical for maintaining species diversity of these ecosystems (Rogers 1979, 2000; Whalley 1996; Osono et al. 2011). New *Xylaria* species and records have been described after recent investigations on wood-rotting fungi in subtropical and tropical forests in China (Ma et al. 2011, 2012, 2013a,b,c). This paper reports a new species of *Xylaria* based on two specimens on the dead tree stump during a survey of xylariaceous fungi in southern China. They differ morphologically

from existing *Xylaria* species, so we describe and illustrate them as a new species.

Materials & methods

Voucher specimens are deposited in the Fungarium of Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China (FCATAS). Microscopical features and measurements were determined from tissues mounted in water and Melzer's iodine reagent. The photographs of asci, ascal apical ring, and ascospores were taken using a Keyence VHX-600E microscope, and photographs of the stromatal surface were taken with a S70 Canon camera attached to a ZSA30w microscope.

Taxonomy

Xylaria nelumboniformis Hai X. Ma, Lar.N. Vassiljeva & Yu Li, **sp. nov.** Figs 1, 2

MycoBank MB 817858

Differs from *Xylaria psamathos* by its tomentose stromata, larger ascospores, and short oblique germ slit.

Type: China, Hainan Province: Diaoluoshan Natural Reserve, 18°46′N 109°50′E, alt. 500 m, on dead stump, 10 Nov. 2012, Haixia Ma, Col. 16 (**Holotype**, FCATAS 547).

Etymology: The epithet refers to the "lotus leaf" shape of the fertile part of the stromata.

Stromata unbranched, upright, with applanate surface of fertile apices, 4–22 mm tall overall, short or long stipitate; fertile parts 4–10 mm diam. × 1.5–3 mm thick, nearly lotus leaf-shaped to irregularly discoid, externally greyish-black to black, with greyish-white sloughing scales and tomentose, especially on the edges; surface plane or with inconspicuous perithecial mounds, finely cracked and tomentose, internally white, woody, texture hard. Stipes 1.5–18 × 1–1.5 mm, terete, inconspicuously tomentose, sometimes contorted, arising from a slightly enlarged base. Perithecia subglobose, 600–900 μm diam. Ostioles slightly prominent. Asci eight-spored, uniseriate, cylindrical, long-stipitate, (180–)190–240(–260) μm long overall, the spore-bearing parts 150–170 × 9–10 μm, the stipes 30–110 μm long, with apical apparatus bluing in Melzer's reagent, urn-shaped to nearly rectangular, (8–)10–12(–15) × 4–5 μm. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral to navicular to crescent-shaped, with narrowly to broadly rounded ends, smooth, (21.5–)23–27.5(–31.5) × (7–)7.5–9(–10) μm, with short oblique germ slit.

Additional specimen examined: **CHINA, Hainan Province: Diaoluoshan Natural Reserve**, 18°46′N 109°50′E, alt. 500 m, on dead stump, 12 Jul. 2013, Haixia Ma (FCATAS 612).

Fig. 1. *Xylaria nelumboniformis* (holotype, FCATAS 547). a–c. Stromata; d, f. Stromatal surface; e. Mature stroma in vertical section. Scale bars: a, c = 6 mm; b = 2 mm; d, e = 1 mm; f = 0.2 mm.

Comments —*Xylaria nelumboniformis* is easily separated from other *Xylaria* species by its lotus-leaf shaped or irregularly discoid flattened stromata that are conspicuously tomentose on the edges. This species is strikingly similar in stromatal morphology to *X. psamathos* Boise, which differs by its smooth

Fig. 2. *Xylaria nelumboniformis* (holotype, FCATAS 547). a. Asci and paraphyses; b. Ascus apical ring; c. Ascospores; d. Germ slit. Scale bars: a = 40 μm; b, c = 10 μm; d = 8 μm.

stromata, its smaller ascospores (11–13 × 4–6 μm) with a straight germ-slit extending the full length of the spore, its flattened-cylindrical ascus rings, and its habitat (dead grass culms in a North Carolina saltmarsh; Boise 1982).

Xylaria nelumboniformis is somewhat similar to *X. crozonensis* P. Leroy & Mornand and *X. michoacana* F. San Martín et al. in stromatal morphology, but *X. crozonensis* has cracked smooth stromata that are narrowly connected to the substrate and smaller ascospores (13.3–15.6 × 7.6–8.8 μm) with a straight inconspicuous germ slit (Leroy & Mornand 2004, Fournier 2014). *Xylaria michoacana* is distinguished by cracked smooth stromata that are narrowly connected to the substrate and slightly smaller subglobose ascospores (21.5–24.5 × 14–16 μm; San Martín et al. 2001).

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Neozygites linanensis sp. nov., a fungal pathogen infecting bamboo aphids in southeast China

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Abstract—*Neozygites linanensis* sp. nov. was identified as infecting the aphid *Takecallis taiwanus* collected on bamboo plants, *Chimonobambusa quadrangularis*, in the Lin'an region of southeast China during spring and summer 2014. This is the first record of a *Neozygites* species on *T. taiwanus*. The Chinese *Neozygites* specimen is described, illustrated, and discussed. The phylogenetic relationship among selected *Neozygites* species is evaluated based on the successful sequence of the SSU rDNA gene from the new fungus.

Key words—natural enemies, entomopathogenic fungi, *Entomophthoromycota,* aphid control, *Neozygitaceae*

Introduction

Bamboo plantations are a common forest type in Asia, and bamboo plants supply food, ethnomedicines, and other necessary materials. Moreover, bamboo forests are extremely helpful for carbon sequestration (Li et al. 2013). Worldwide, over 40 aphid species infest bamboos, and many bamboo aphids with robust reproductive and dispersal abilities cause considerable loss of bamboo productivity (Blackman & Eastop 1994). Zhou et al. (2014) recently reported on one entomophthoralean aphid pathogen, *Conidiobolus obscurus*

(I.M. Hall & P.H. Dunn) Remaud. & S. Keller, that infected four bamboo aphid species in the laboratory. However, rarely has information been recorded about the natural enemies and pathogens of bamboo aphids and their interaction in the field. Recently entomopathogenic fungi on bamboo aphids were surveyed in China's Lin'an region, an important bamboo forest area. Mycosed individuals of one widespread bamboo aphid species that frequently infects local bamboo plants, *Takecallis taiwanus* (Blackman & Eastop 1994), were observed during spring and early summer. Initial morphological examination of the infected *T. taiwanus* showed that the entomopathogenic fungus represented *Neozygites*. The only previously reported aphid-pathogenic *Neozygites* species in China is *Neozygites fresenii* (Nowak.) Remaud. & S. Keller, which has been found on *Aphis gossypii* in northern China and on *Myzus persicae* on cabbage in southern China (Zhang et al. 1983, Huang et al. 2008).

Research on *Neozygites* species in natural ecosystems has been limited, particularly in comparison to that reported worldwide for other aphid-specific entomophthoralean species in *Pandora*, *Conidiobolus*, *Entomophthora*, and *Zoophthora* (Barta & Cagáň 2006, Keller 2006). Ben-Ze'ev (1981) reported *Neozygites turbinata* (R.G. Kenneth) Remaud. & S. Keller in Israel on the aphid *Pterochloroides persicae*. *Neozygites lageniformis* (Thaxt.) Remaud. & S. Keller was found on aphids *Myzocallis coryli* on *Corylus avellana* in Chile (Barta & Cagáň 2006). In Switzerland, Keller reported *Neozygites cinarae* S. Keller on *Cinara pilicornis* on *Picea abies* (Keller 1997) and described *Neozygites remaudierei* S. Keller from *Myzocallis coryli* (Keller 2006). Barta (2009) reported eighteen species of aphids on woody plants infected by *N. fresenii* and one species infected by *Neozygites cinarae* in the Mlyňany arboretum in Slovakia. Montalva et al. (2013) discovered a new species, *Neozygites osornensis* Montalva & Barta, on Cinara aphids associated with *Cupressus macrocarpa* and *Austrocedrus chilensis* in Chile. Other aphid-pathogenic species reported in agroecosystems include *Neozygites microlophii* S. Keller on nettle aphids in Switzerland and *Neozygites lecanii* (Zimm.) Ben Ze'ev & R.G. Kenneth on *Lecanium viride* in South East Asia.

Additionally, gene-based research on *Neozygites* species is scarce, which further hinders understanding of the phylogenetic relationships and the status of this genus within the entomophthoroid fungi. Finally, difficulty in obtaining in vitro cultures and its infrequent collection makes *Neozygites* a much-understudied genus (Humber 2012a). The aim of the present work was to identify the *Neozygites* species collected on mycosed *T. taiwanus* populations in bamboo forests through morphological and molecular analyses. For sequencing fungal

DNA from resting spores within host cadavers, we conducted a subcloning procedure that is widely used to diagnose mixed sequences in samples (Pérez-Tris & Bensch 2005). We then used the sequence of the small subunit ribosomal RNA (SSU rRNA) gene obtained from the mycosed *T. taiwanus* specimens to analyze the genetic relationships of other *Neozygites* species.

Materials & methods

Field collection & material processing

The survey of local bamboo–*Chimonobambusa quadrangularis* forests in the suburb of Lin'an city, Zhejiang province, China, was carried out during the spring and summer of 2014. Specimens of dead mycosed adults of *T. taiwanus* attached to bamboo leaves were carefully removed and transferred to a paper bag and then stored in a polystyrene cooler at –20°C (Benjamin et al. 2004). The fungal pathogen (N51) was cultured in vivo by host passage in leaf-containing Petri dishes in the laboratory at 20–24°C. Two types of solid culture medium were tested during our attempts to let the fungal spores eject on the surface of medium and obtain in vitro culture of this fungus: (i) Sabouraud dextrose agar plus yeast extract (SDAY: 4% glucose, 1% peptone, 1% yeast extract, and 1.5% agar) and (ii) SEMA (80% SDAY plus 11.5% egg yolk, 8.5% pure milk) were attempted to cultivate the fungus (Hajek et al. 2012).

Morphological evaluations

The fungus was identified based on morphological characteristics according to Keller (1991, 1997) and Humber (2012a). Semi-permanent slide mounts were prepared in lactophenol-aceto-orcein (LPAO) as described by Keller (1987) and deposited in the Forest Insect and Disease Herbarium, Zhejiang Agricultural and Forestry University, Lin'an, China (ZAFU). Fungal structures were examined with a Zeiss AX10 microscope, photographed with a Zeiss HAL100 digital camera at a magnification of 400×, and measured with the software Zen 2012 (Zeiss). The measurements were based on 50 objects per microstructure. For each microstructure, mean value of measurements, standard error of the mean (± SEM), and maximal and minimal values were calculated, cited below as minimum–mean±SEM–maximum.

The fungal material obtained from *T. taiwanus* in China was microscopically compared with *Neozygites fresenii* attacking *Rhopalosiphum padi* in Poland (where the fungus *N. fresenii* was first described) and *N. fresenii* from Slovakia, Austria (European material was provided by Dr. Marek Barta), and Chile. Two other aphid-pathogenic *Neozygites* species, *N. osornensis* and *N. cinarae*, were obtained during a survey of natural enemies of tree dwelling aphids in southern Chile.

DNA extraction & PCR amplification

Resting spores of the *Neozygites* isolates for DNA extraction were obtained from laboratory-infected *T. taiwanus* bamboo aphids. Fresh mycosed cadavers collected from the field were placed in aphid cohorts in leaf-containing dishes at 20–24°C. After three days of incubation, new cadavers, including black cadavers with resting

Table 1. Morphological comparison of *Neozygites linanesis* with *N. fresenii*, *N. microlophii*, and *N. remaudierei*

Species	Country (source)	Host(s)	Hyphal bodies diam. (µm)	Primary conidia		Capilliconidia		Zygospores	
				L (µm)	W (µm)	L (µm)	W (µm)	L (µm)	W (µm)
Neozygites linanesis	China	*Takecallis taiwanus*	14.5–21	18.8–26.5	14.4–22.8	17.3–29.3	10.6–16.2	21.6–37.2	15.5–26.9
			(10)		(7)		(7)		(2)
Neozygites fresenii	Poland (Bałazy 1993)	3 genera	14–18	18–20	15–18	19–27	11–14	24–49	16–30
	Switzerland (Keller 1991)	3 genera	14–18 (4)	16–24	12–21	16–33	9–17	25–48	17–24
	Slovakia (Barta 2004)	15 genera	—	13.9–25.7	11.9–19.8	15.8–31.7	9.9–19.8	36–46.8	19.8–25.2
Neozygites microlophii	Switzerland (Keller 1991)	*Microlophium carnosum*	17–22	21–30	15–22	19–29	12–19	29–50	17–27
			(5)		(5)				
Neozygites remaudierei	Switzerland (Keller 1991)	*Myzocallis coryli*	17–23	21–25	17–23	24–36	12–18	—	—

Nuclei per structure (in parentheses); L = length; W = width

spores, were found in each dish. Cadavers with resting spores were collected, placed into 1.5 mL tubes (50 cadavers per tube), and stored at 4°C. DNA extraction followed the protocol set forth in the TakaRa (Ver. 5.0) MiniBest universal genomic DNA extraction Kit. To maximize DNA extraction from the resting spores, several 2.5 mm sterile zirconia beads were added into each tube and then vibrated for 1 min, repeated several times during the 56°C water bath for cell lysis. The extracted DNA concentration was quantified by Nanodrop® ND-1000 and maintained at –20°C.

Universal fungal primers NSR 0004-5' (5'-CTGGTTGATTCTGCCAGT-3'; Gargas & DePriest 1996) and SR13 (5'-AATGATCCTTCCGCAGGT-3'; Depress 1993) were used to amplify the nuclear SSU ribosomal RNA gene. PCR was carried out with 30 µl per sample and in duplicates. Each sample contained 2 × PrimeSTAR® Max Premix, 0.12 µM of each primer, sterile distilled water, and 2 µl template (25 ng/µl of fungal DNA isolated). The thermal profile of the 35 cycles of PCR consisted of the initial step at 94°C for 3 min, followed by 35 cycles at 94°C for 15 s, 55°C for 15 s and 72°C for 10 s and the final step at 72 °C for 5 min. PCR products were run in 1% agarose gels with 0.1 µl/mL of ethidium bromide in 1 × TBE buffer at 100 V for 30 min. The PCR products were examined and photographed on a UV light transilluminator.

DNA sequencing & analysis of SSU rDNA sequences

The amplified DNA was sent to TaKaRa Biotechnology (Dalian, China) for TA cloning and sequencing. The target band of ~1.5 kb was recovered, and the DNA fragment was ligated into a pMD18-T vector (TaKaRa, China) after adenine additions. *Escherichia coli* JM109 cells were transformed and spread onto plates of Luria-Bertani agar plus X-Gal, IPTG and Ampicillin. The plates were incubated overnight at 37°C. Three positive colonies (white color) were selected through blue-white screening as replicates. The vector primers M13-47 (5'-CGCCAGGGTTTT CCCAGTCACGAC-3') and RV-M (5'-GAGCGGATAACAATTTCACACAGG-3') were used to amplify the SSU rRNA gene sequence of the *Neozygites* isolate, which was inserted into the pMD18-T plasmid in monoclonal colony PCR. And then the PCR products from each clone were sequenced. Copies of the *Neozygites* sp. (N51) sequences were pairwise compared with other *Neozygites* species online (ClustalW2, the European Bioinformatics Institute, Cambridge, UK). A multiple alignment of sequences was performed with ClustalX 2.0 (Larkin et al., 2007) among SSU rDNA sequences, and a dendrogram was constructed with Seaview 4.0 (Gouy et al. 2010) using the neighbor-joining method with 1000 bootstraps. The sequences obtained in this analysis and from GenBank for other *Neozygites* species such as *N. osornensis* (KC822922), *N. cinarae* (KC822923), *N. turbinata* (KC822924), *N. parvispora* (MacLeod & Carl) Remaud. & S. Keller (AF296760), *N. floridana* (J. Weiser & Muma) Remaud. & S. Keller (AF296758), *N. tanajoae* Delal. et al. (AY233981), and *Neozygites* sp. (AF296759) obtained from GenBank were included in the analysis. Other species infecting aphids on bamboo plants as *Conidiobolus obscurus* (JQ014014) (Zhou et al. 2014) were chosen as outgroup in the phylogenetic tree.

Results

During the survey of natural enemies of the bamboo aphid, mycosed cadavers of *T. taiwanus* were observed from spring to summer 2014. Grey-brown infected aphids were easily recognized hanging on the lower sides of new bamboo leaves. Microscopic observations of the cadavers revealing fungal infection by *Neozygites* represented the first find of mycosed bamboo aphids in Lin'an city, southeast China.

Cultural & morphological observations

No in vitro cultures were successfully isolated from infected individuals, despite attempts to isolate the entomopathogenic fungi using two different solid culture media.

Measurements of taxonomically important structures and comparison with morphologically similar species are presented in Table 1. *Neozygites* obtained from *T. taiwanus* in China was morphologically distinguished from the other aphid *Neozygites* species mainly by higher number of nuclei per hyphal body, primary conidia, and capilliconidia (Plate 1). The sizes of the major morphological microstructures in the Chinese fungus are larger than in *N. fresenii* from Austria and Slovakia, similar in *N. fresenii* from Poland and Chile, and smaller than in *N. osornensis* and *N. cinarae* from Chile (except for a larger capilliconidial diameter in the Chinese *Neozygites* specimens).

Molecular analysis

The fungal SSU rDNA gene from the Chinese *Neozygites* fungus was successfully sequenced and its sequence (KM386989) deposited in the GenBank database (NCBI). Pairwise comparisons of the 1524 bp long aligned SSU rDNA gene sequence showed that the Chinese fungal sequence shares a 92% similarity with *N. turbinata* and a 93% similarity with *N. cinarae* and *N. osornensis*—all species infecting aphid hosts. On the other hand, the Chinese *Neozygites* sequence matched only 73% with *N. tanajoae*, 75% with *N. floridana*, and 77% with *N. parvispora,* all species affecting only mites or thrips. Furthermore, the phylogenetic neighbor-joining SSU rDNA tree separates the bamboo aphid fungus from the clade containing other aphid-associated *Neozygites* species, supporting the fungus found on *T. taiwanus* as an independent species within *Neozygites* (Plate 2).

Taxonomic description

Neozygites linanesis X. Zhou & C. Montalva, **sp. nov.** Plate 1

MycoBank 814659

Differs from *Neozygites fresenii* by its larger hyphal bodies and primary conidia (both containing a greater number of nuclei) and by its smaller zygospores.

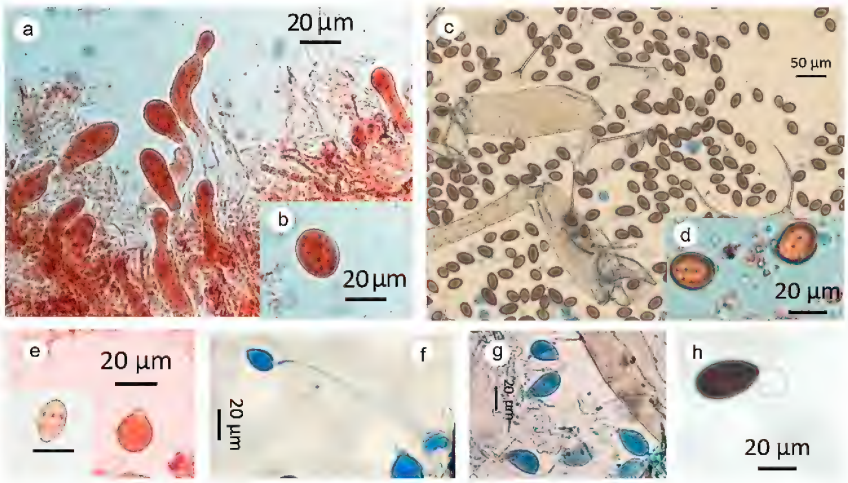

Plate 1. Fungal structures of *Neozygites linanesis* on bamboo aphid *Takecallis taiwanus*. a. Unbranched conidiophores. b. Subspherical hyphal body with visible nuclei. c. Maturing resting spores filling cadaver. d. Resting spores with two visible nuclei. e. Primary conidium. f. A developing capilliconidium on a long slender capillary tube. g. Mature capilliconidia adhered to host leg parts. h. Resting spore developing by conjugation of two spherical hyphal bodies. [Figures a, b, d, e, h stained in lactophenol-aceto-orcein; figures c, f, g in cotton blue.]

Type: China, Zhejiang Province, suburb of Lin'an city, 30°15′49″N 119°44′23″E, from *Takecallis taiwanus* (Takakashi) on *Chimonobambusa quadrangularis* (Fenzl) Makino, 20 May 2014, coll. Xiang Zhou (**Holotype**, ZAFU IP15091101; GenBank KM386989),

Etymology: The specific epithet refers to the name of the type locality.

Hyphal bodies spherical to subspherical, 14.5–17.4±1.6–21 µm in diameter, containing an average of 10 nuclei (1.9 ± 0.6 µm diam.); primary conidia spherical, 18.8–22.9±1.8–26.5 × 14.4–17.6±1.9–22.8 µm with a distinct cylindrical papilla, containing an average of 7 nuclei; capilliconidia forming from primary conidia, almond-shaped, 17.3–23.3±2.3–29.3 × 10.6–13.6±1.6–16.2 µm, containing an average of 7 nuclei, borne on a long slender capillary tube 66.5 ± 15.8 µm; zygospores broadly ellipsoidal, thick-walled, with a black, smooth episporium, 21.6–28.1±3.7–37.2 × 15.5–19.2±2.3–26.9 µm. Rhizoids not observed.

Observed in spring and summer 2014 in just one locality in China.

Discussion

The genus *Neozygites*, which differs from the other entomophthoroid fungal species by the nuclear structure and behaviour during mitosis (Butt & Humber 1989), currently comprises 19 entomopathogenic fungal species that

may eventually be split into two genera differentiated by their host affinities and resting spore morphologies (Humber 2012b). The nine aphid-pathogenic species (Keller 2006, Montalva et al. 2014a) have smooth ovoid resting spores, and none of these species (including the type species, *N. fresenii*) has been grown in vitro; the remaining 10 species infect primarily mites (but include some species on non-aphid insect hosts) and produce rough globose zygospores; all in vitro cultures of *Neozygites* species have been isolated from mites.

From the morphological point of view, *N. fresenii* and *N. linanesis* are closely related. *Neozygites fresenii* is a widely distributed fungal pathogen of aphids (Milner & Holdom 1986, Hatting et al. 1999, Barta & Cagáň 2006, Scorsetti et al. 2007). It was named and described as *Empusa fresenii* by Nowakowski (1883), who first discovered the fungus in naturally infected aphids collected in Poland. This is the first described member of the *Neozygites* genus. While both fungi are pathogenic to aphids, *N. linanesis* can be distinguished from *N. fresenii* mainly by its greater average number of nuclei per hyphal body and primary conidium. Also, the hyphal bodies and primary spores in *N. linanesis* are somewhat larger than those in *N. fresenii* recorded in the literature from Poland, Switzerland, and Slovakia, while its zygospores are slightly small compared those recorded for *N. fresenii* (Keller 1991, Bałazy 1993, Barta 2004). Compared to other aphid-pathogenic *Neozygites* species, all the recorded dimensions for microstructures in *N. microlophii* and *N. remaudierei* are larger than those in *N. linanesis* (Keller 1991, 2006).

Unfortunately, there are no DNA sequences from *N. fresenii* available for molecular comparisons with other *Neozygites* species. Nonetheless, SSU rDNA gene analysis from *N. linanesis* and the other aphid-pathogenic *Neozygites* species showed clearly that *N. linanesis* is separated in a different clade from *N. cinarae*, *N. turbinata*, and *N. osornensis* (Plate 2); this analysis, when combined with the morphological differences, suggests that *N. linanesis* is a distinct species within *Neozygites*.

Moreover, the molecular differences between aphid- and mite-/thrip-pathogenic *Neozygites* species are consistent with the proposal by Humber (2012b). SSU rDNA gene-based analysis successfully separated *N. linanesis* from a morphologically similar *Neozygites* species, thus supporting the morphological observations made in this study. This gene has been previously used to separate *N. tanajoae* from the morphologically similar *N. floridana* (Delalibera & Humber 2004) as well as *N. osornensis* from *N. turbinata* and *N. cinarae* (Montalva et al. 2014b). We regard the SSU rDNA gene as a valid tool for identifying *Neozygites* species.

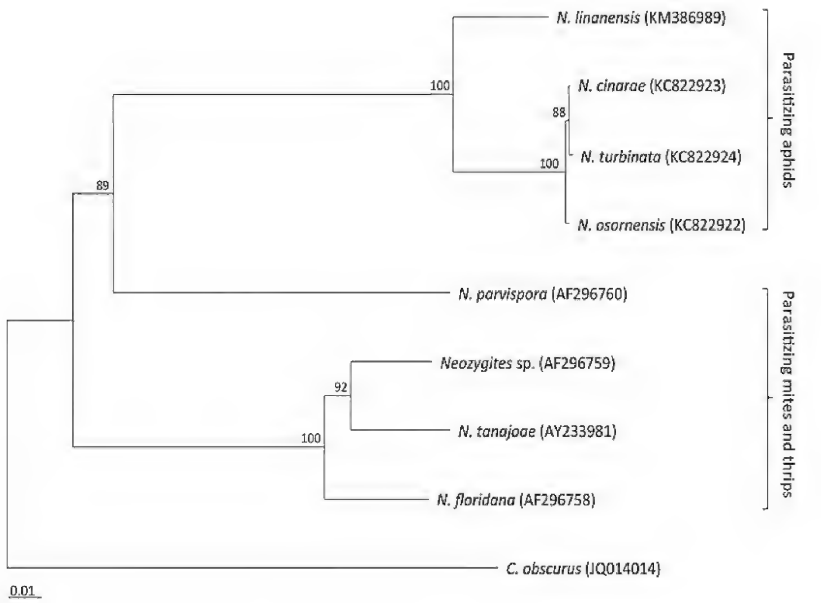

Plate 2. Dendrogram showing the relationships among SSU rDNA sequences of *Neozygites linanesis* isolated from bamboo aphid *Takecallis taiwanus* and other DNA sequences of *Neozygites* species published in GenBank. *Conidiobolus obscurus* was used as outgroup and the numbers on each branch indicate bootstrap values (1000 replicates).

In this work we have described a new *Neozygites* species that can infect *Takecallis* aphids and observed the spread of *N. linanensis* in host populations during the aphid infestation period. *Neozygites linanesis,* which appears to be a natural control agent of *T. taiwanus* in bamboo forests in China, represents the first record of a *Neozygites* species associated with *T. taiwanus* populations. Our 2014 surveys also revealed that *N. linanesis* infects other aphid species in China, such as *Metamacropodaphis bambusisucta.* The identification of *N. linanesis* and the sequencing of its SSU rDNA gene have thereby added to the knowledge regarding natural fungal enemies of bamboo aphids.

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Five *Lecidea* lichens new to China

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Abstract—Five *Lecidea* lichen taxa—*L. andersonii*, *L. grisella, L. laboriosa*, *L. atrobrunnea* subsp. s*axosa*, *L. atrobrunnea* subsp. *stictica*—are reported for the first time from China.

Keywords—Asia, *Lecidiaceae*, *Lecideales*, saxicolous lichens, taxonomy

Introduction

The lichen genus *Lecidea* Ach. (*Lecideaceae*) was originally described by Acharius (1803). In the sense of Zahlbruckner, the genus once represented one of the largest lichen genera and included about 1200 species (Schmull et al. 2011). Of the 427 species included in *Lecidea* sensu lato (Kirk et al. 2008), only about 100 (all saxicolous) are accepted in *Lecidea* sensu stricto, which is characterized by a *Lecidea*-type ascus (Hertel 2006, Smith et al. 2009).

During our study of lecideoid lichens from China, five *Lecidea* taxa were recognized as new to the country—*L. andersonii, L. grisella, L. laboriosa, L. atrobrunnea* subsp. *saxosa*, and *L. atrobrunnea* subsp. *stictica*.

Materials & methods

The specimens studied are preserved in either the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) or the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN). The

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specimens were examined using standard microscopic techniques and hand-sectioned under a Nikon SMZ 645 dissecting microscope. Anatomical descriptions were based on observations of these preparations under a Nikon EclipseE200 microscope. Sizes cited for the thallus, apothecium, hymenium, and exciple are based on five measurements per specimen. Ascospore dimensions are based on ten measurements per specimen and presented as a range, with outlying values given in parentheses. Secondary metabolites of all the specimens were identified using TLC and solvent C (Orange et al. 2010). Medullas were tested for amyloid reactions using IKI (10% aqueous potassium iodide). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Lecidea andersonii Filson, Muelleria 3(1): 16 (1974). FIG. 1

THALLUS often poorly developed, rimose to areolate, angular and usually appressed along rock crevices, whitish to grey; medulla I+ intensively violet-blue; prothallus absent. APOTHECIA sessile, constricted at the base, round to irregular, (0.4–)1–1.4 mm diam.; disc black, flat to slightly convex, mostly epruinose, rarely with a thin whitish pruina; margin distinct, sometimes becoming flexuose. Exciple hyaline, outer margin dark green to brownish green, 90–150 μm wide; epihymenium dark green; hymenium hyaline, 50–80 μm high; hypothecium brownish, with crystals; paraphyses simple, coherent, with swollen apices. ASCI clavate; 8-spored; ascospores narrowly ellipsoid, (8–)9–12 × 3.5–4.4 μm. PYCNIDIA not observed.

CHEMISTRY—Cortex and medulla K–, C–, KC–. 4-O-demethylplanaic acid was detected by TLC.

SUBSTRATE—On siliceous rocks.

DISTRIBUTION—*Lecidea andersonii* has been reported from Europe and Antarctica (Hertel et al. 2009, Ruprecht et al. 2010). New to China.

SPECIMENS EXAMINED: **CHINA. XIZANG**, Anduo, Mt. Tanggula, alt. 5200 m, on rock, 30 Jul. 2011, Y.L. Cheng 20118836A, 20116422, 20116396, 20116343, 20116395, 20126439 (SDNU); **XINJIANG** Bukang, Mt. Tianshan, alt. 3700 m, on rock, 17 Aug. 2012, L.L. Zhang 20129086, 20129092, 20129135 (SDNU); **SICHUAN**, Litang, Mt. Kazila, alt. 4700 m, on rock, 7 Nov. 2008, Z.J. Ren 20080005, 20108491, 20080159 (SDNU).

COMMENTS—*Lecidea andersonii* is morphologically similar to *L. diducens*, also with an endolithic thallus, sessile apothecia, dark green epihymenium, brownish hypothecium, and narrowly ellipsoid ascospores, but *L. diducens* is distinguished by an I– medulla and C+ red exciple and contains 2'-*O*-methylanziaic acid (Inoue 1991).

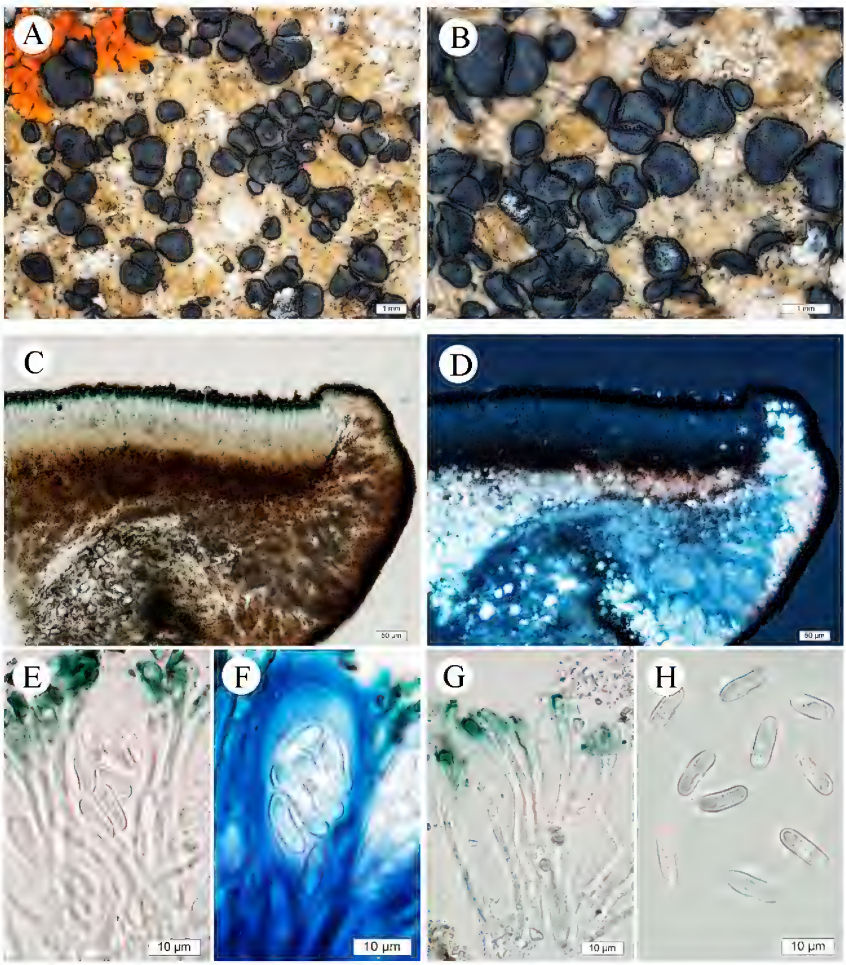

Fig. 1. *Lecidea andersonii* (20129135, SDNU). A: thallus; B: apothecia; C: apothecium section; D: exciple and hypothecium with crystals; E: ascus and ascospores; F: amyloid reaction of ascus; G: paraphyses; H: ascospores.

Lecidea grisella Flörke, Lich. Schlesien: nos 141–142 (1829). Fig. 2

Thallus contiguous, cracked, rimose, rarely areolate, whitish grey to pale grey-brown, generally thicker and never convex; medulla I–; prothallus absent. Apothecia black, immersed, ≤0.6–1.2(–1.5) mm diam., flat to slightly concave; margin distinct, regular. Exciple dark brown, with crystals; epihymenium olive-

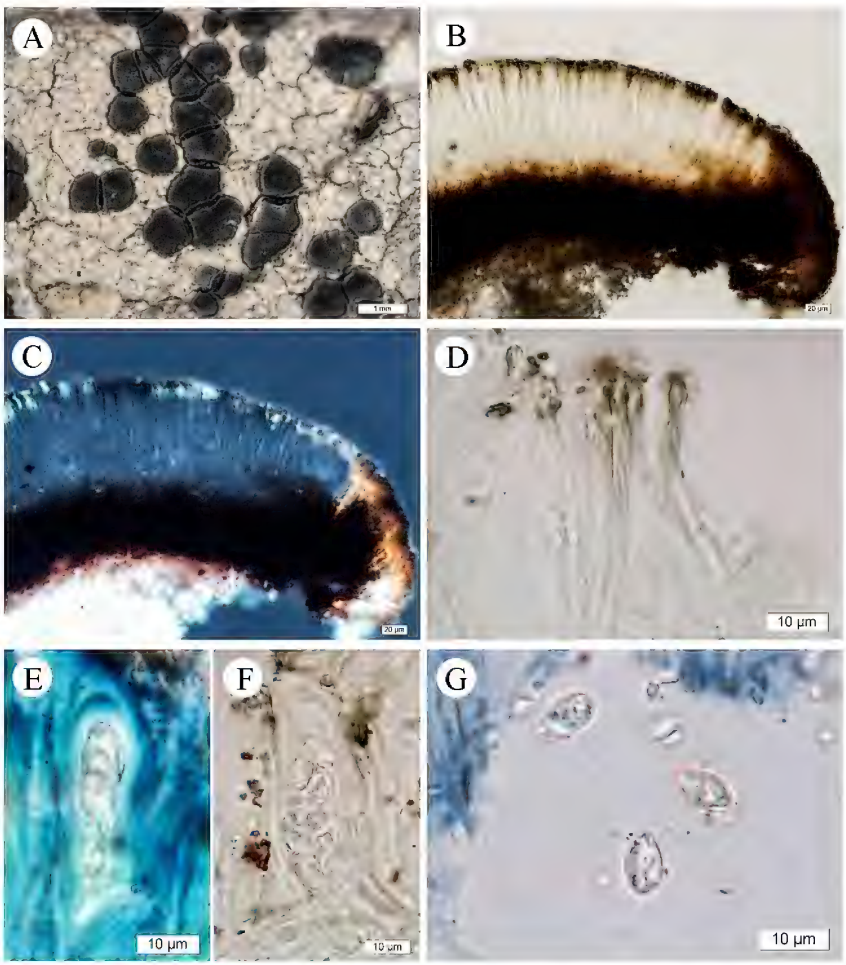

Fig. 2. *Lecidea grisella* (YN182, SDNU). A: thallus; B: apothecium section; C: exciple and hypothecium with crystals; D: paraphyses; E: amyloid reaction of ascus; F: ascus and ascospores; G: ascospores.

brown, with little crystals; hymenium colorless, 60–80 µm; hypothecium dark brown, without crystals; paraphyses 2–3.5 µm thick. Asci clavate; ascospores ellipsoid, simple, 8–12(–13) × 5–6(–7) µm. Pycnidia not observed.

Chemistry—Medulla K–, C+ red, KC+ red; exciple C+ red. Chemical constituent: gyrophoric acid.

Substrate—On siliceous rocks.

Distribution—*Lecidea grisella* has been reported from Asia, Africa, and Europe (Smith et al. 2009). New to China.

Specimens examined: **China. Yunnan**, Tangdan, Lanniping, alt. 3800 m, on rocks, 14 May. 2014, W.C. Wang YN222, YN227, YN226, YN225, YN252; L. Hu YN185, YN181, YN182, YN183 (SDNU).

Comments —*Lecidea grisella* is similar to *L. fuscoatra*. The main difference between the two species is visible at the margins: *L. fuscoatra* forms as an areolated thallus on a prothallus, whereas *L. grisella* forms with a continuous thallus that becomes rimose or splits secondarily up into areoles that tend to remain flat with angular edges for a long time (Aptroot & Herk 2007). *Lecidea fuscoatra* occurs on a wider variety of substrata (rather smooth, slightly nutrient-rich siliceous rocks, walls, and brick) and, unlike *L. grisella,* can be found growing on roof tiles and (rarely) on timber (Smith et al. 2009).

Lecidea laboriosa Müll. Arg., Flora 57: 187 (1874). Fig. 3

Thallus endolithic, rarely epilithic; medulla white, I–; prothallus absent. Apothecia black, loosely attached with strongly constricted base, 0.6–1.4 mm diam; disc black, flat to convex, epruinose or faintly pruinose; margin black, persistent, regular to flexuose. Exciple unpigmented or sometimes pale brown, peripherally dark brown, 30–80 µm wide, with crystals; epihymenium black green, olive or brown; hymenium colorless or more rarely with a pale violet tinge, 37.5–60 µm tall, I+ blue; subhymenium colorless, 25–50 µm thick; hypothecium colorless to pale brown, with crystals; paraphyses simple, rarely branched, anastomosed, apical cells 3.5–5 µm wide, globose, with dark caps. Asci clavate, 8-spored; ascospores oblong, 7–11.5 × 2.5–4 µm. Pycnidia not observed.

Chemistry—Cortex and medulla K–, C–, KC–. 4-O-demethylplanaic acid was detected by TLC.

Substrate—on acidic rocks.

Distribution—*Lecidea laboriosa* has been reported from Asia, Europe, and North and South America (Hertel et al. 2009, Nash et al. 2004). New to China.

Specimens examined: **China. Xinjiang**, Ursula, Bayingou, alt. 2100 m, on rock, 23 Jul. 2013, C. Chao XL0239 (SDNU); Aletai, Buerjin, Bulanai, alt. 1920 m, on rock, 23 Jul. 2013, Q. Ren 2012607 (SDNU); Yili state, Qiongbola Qapqal national Forest Park, alt. 1861 m, on rock, 12 Jun. 2014, P.M. Wang 20140152 (SDNU); **Sichuan**, Daocheng, Mt. Haizi, alt. 4390 m, on rock, 19 Sep. 2002, L.S. Wang 02–21602 (KUN).

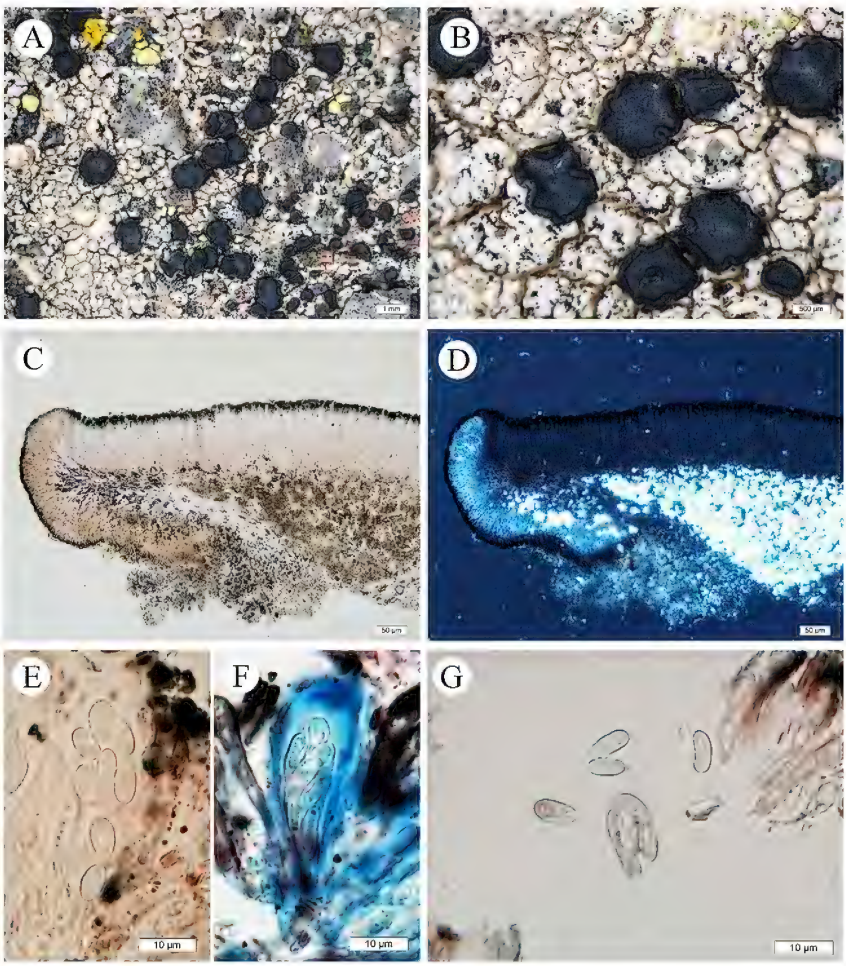

Fig. 3. *Lecidea laboriosa* (2012607, SDNU). A: thallus; B: apothecia; C: apothecium section; D: exciple and hypothecium with crystals; E: ascus and ascospores; F: amyloid reaction of ascus; G: ascospores.

Comments—*Lecidea laboriosa* is morphologically similar to *L. auriculata*, which is distinguished by a K+ red-brown exciple, I+ violet-blue medulla, and the presence of confluentic acid (Nash et al. 2004).

Lecidea atrobrunnea* subsp. *saxosa Hertel & Leuckert,
Lich. Flora Gr. Sonoran Desert Region 2: 296 (2004). Fig. 4

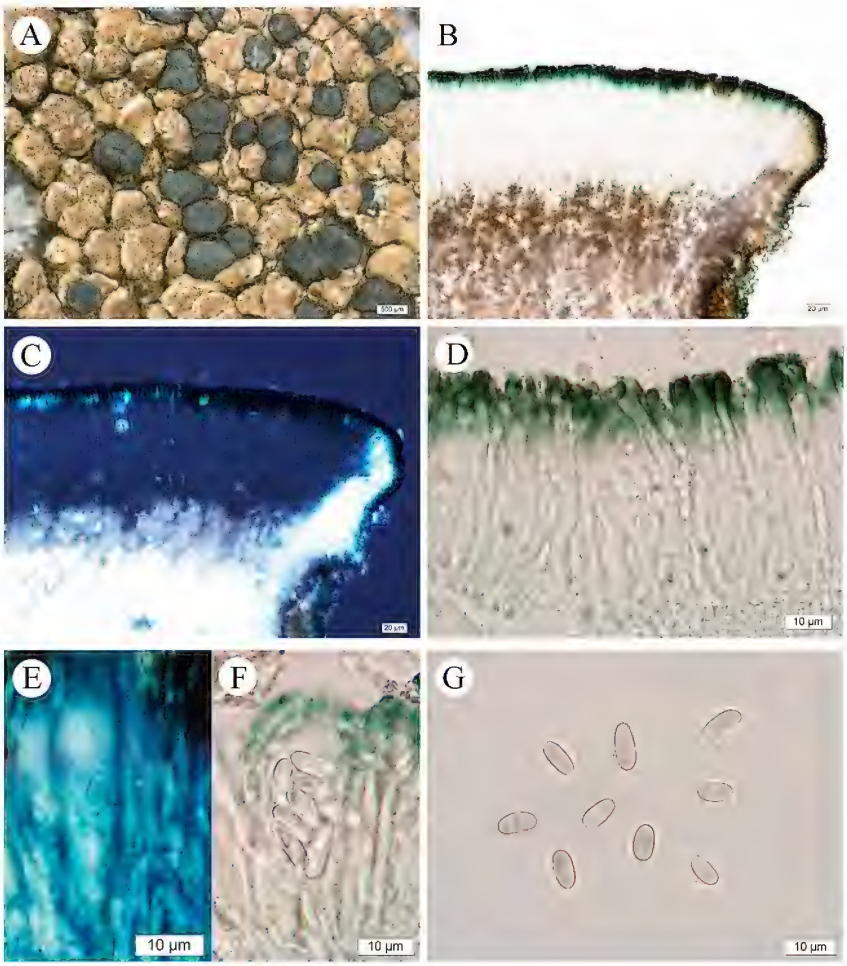

Fig. 4. *Lecidea atrobrunnea* subsp. *saxosa* (20140920, SDNU). A: thallus and apothecia; B: apothecium section; C: exciple and hypothecium with crystals; D: paraphyses; E: amyloid reaction of ascus; F: ascus and ascospores; G: ascospores.

Thallus crustose, bullate to subsquamulose, continuous, brown to dark brown, polished, with an epinecral layer, 5–30 µm, areolate; medulla I+ violet-blue, hypothallus black. Apothecia black, epruinose, ≤1.6 mm diam., adnate, between or partly on the areolae, moderately constricted at the base; with a somewhat prominent margin. Exciple with a greenish black, epihymenium-like rim, unpigmented interior; epihymenium green or olive-green; hymenium

40–60 µm tall; subhymenium hyaline 15–40 µm tall; hypothecium hyaline to pale ochre; paraphyses simple, anastomosing, with swollen apices. Asci clavate, 8-spored; ascospores hyaline, ellipsoid-oblong, simple, 7–10 × 3.5–4 µm. Pycnidia not observed.

Chemistry—Cortex and medulla K+ red, C–, KC–. Norstictic acid was detected by TLC.

Substrate—on siliceous rocks.

Distribution—*Lecidea atrobrunnea* subsp. *saxosa* has been reported from Asia, Europe, North America, and Antarctica (Nash et al. 2004). New to China.

Specimens examined: **CHINA. Xinjiang**, Gongnaisi, Panchen Ditch, alt. 3800 m, on rock, 3 Jul.2014, P.M. Wang 20140675A (SDNU); Hucheng, Kurt, alt. 3700 m, on rock, 12 Jul.2014, P.M. Wang 20140950, 20140920 (SDNU).

Comments—*Lecidea atrobrunnea* subsp. *saxosa* is similar to *L. atrobrunnea* subsp. *atrobrunnea*; but subsp. *saxosa* contains norstictic acid while subsp. *atrobrunnea* contains confluentic acid (Nash et al. 2004).

Lecidea atrobrunnea* subsp. *stictica Hertel & Leuckert,
Lich. Flora Gr. Sonoran Desert Region 2: 297 (2004). Fig. 5

Thallus crustose, bullate to subsquamulose, continuous, brown to dark brown, polished, with an epinecral layer, 5–30 µm, areolate; medulla I+ violet-blue, hypothallus black. Apothecia black, epruinose, ≤1.7(–2.5) mm diam., adnate, between or partly on the areolae, moderately constricted at the base; with somewhat prominent margin. Exciple with a greenish black, epihymenium-like rim, unpigmented inside; epihymenium bright green, olive-green, to green-brown; hymenium 40–60 µm tall; subhymenium hyaline 15–60 µm tall; hypothecium hyaline to medium brown (rarely dark brown); paraphyses simple, anastomosing, with swollen apices. Asci clavate, 8-spored; ascospores hyaline, ellipsoid-oblong, simple, 7–11 × 3–4.5 µm. Pycnidia not observed.

Chemistry—Cortex and medulla K–, C–, KC–. Stictic acid and hypostictic acid was detected by TLC.

Substrate—On siliceous rocks.

Distribution—*Lecidea atrobrunnea* subsp. *stictica* has been reported from Asia, Europe, North America, and Antarctica (Nash et al. 2004). New to China.

Specimens examined: **CHINA. Xinjiang**, Hami, Baishitou, alt. 2800 m, on rock, 28 Jul. 2013, C.Li XL0111 (SDNU); Gongnai, Panchen Ditch, alt. 3800 m, on rock, 3 Jul. 2014, P.M. Wang 20140675B (SDNU).

Comments—*Lecidea atrobrunnea* subsp. *stictica* is morphologically similar to *L. atrobrunnea* subsp. *atrobrunnea* and *L. atrobrunnea* subsp. *saxosa*, but subsp. *stictica* contains stictic acid or hypostictic acid (Nash et al. 2004).

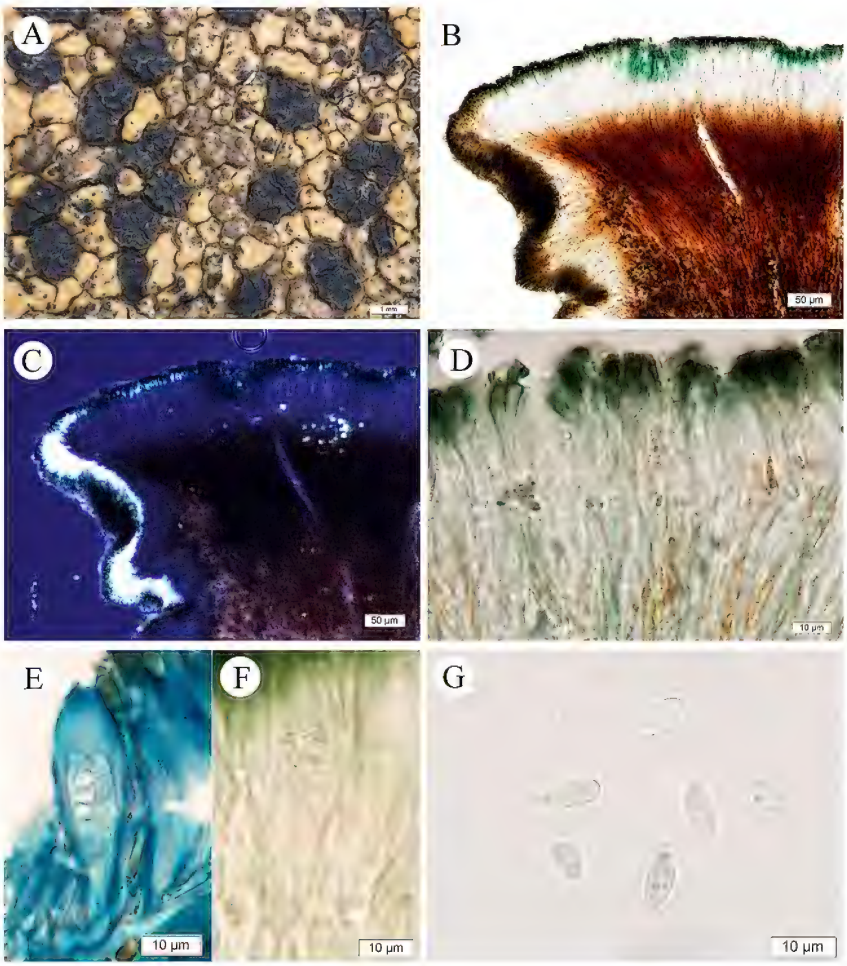

Fig. 5. *Lecidea atrobrunnea* subsp. *stictica* (20140675B, SDNU). A: thallus and apothecia; B: apothecium section; C: exciple with crystals; D: paraphyses; E: amyloid reaction of ascus; F: ascus and ascospores; G: ascospores.

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Pseudobaeospora lilacina sp. nov., the first report of the genus from China

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Abstract—A new species, *Pseudobaeospora lilacina*, is described based on materials collected from Guangdong Province, China. The fungus represents the first record of the genus in China. The new species is characterized by its lilac pileus, stubbly stipe, and ellipsoid spores. Phylogenetic analyses of the internal transcribed spacer regions (ITS) and nuclear ribosomal RNA gene large subunit (nrLSU) support *P. lilacina* within *Pseudobaeospora* and as distinct from all other sequenced species of the genus. The combination of morphological and molecular data confirmed the fungus to be new. The new species is compared to morphologically similar species and its taxonomic position is discussed.

Key words —*Agaricales, Basidiomycota,* taxonomy, *Tricholomataceae*

Introduction

Pseudobaeospora Singer was erected by Singer (1942) and emended by Bas (2003); the genus is characterized by small basidiocarps, pale to dark lilac pilei, and small spores. Bas (2003), who described 25 *Pseudobaeospora* species, divided the genus into five groups (Albidula, Celluloderma, Pyrifer, Frieslandica, and Pillodii) based primarily on basidiocarp color and the presence or absence of clamp connections. Kirk et al. (2008) estimated about 20 species for the genus, while the current Index Fungorum database (http://www.index fungorum.org accessed June 2016) lists 34 published taxa.

The type species of the genus, *Pseudobaeospora oligophylla* (Singer) Singer, was originally found in the Altai Mountains of central Asia by Singer (1938; as *Baeospora oligophylla*). *Pseudobaeospora* species were subsequently described from Asia, Europe, and North and South America (Singer 1986). Additional species have been described from California (Vellinga 2001, 2009; Schwarz 2012), Hawaii (Desjardin et al. 2014), and Slovakia (Adamčík & Jančovičová 2011), but the genus remained unreported from China.

The taxonomic position of *Pseudobaeospora* has been ambiguous. Singer (1942) initially placed the genus in *Tricholomataceae* as an independent genus but soon afterwards transferred it to *Agaricaceae*, at first in tribus *Lepioteae* (Singer 1951) and later in tribus *Cystodermateae* (Singer 1986). However, Kühner (1980) retained the genus in *Tricholomataceae*, a placement supported by subsequent researchers (Bas 2003, Desjardin et al. 2014).

Here we describe a new species of *Pseudobaeospora* from China based on a combination of morphological and molecular data; we also compare the morphological similarities and differences between the new *P. lilacina* and previously described *Pseudobaeospora* species.

Material & methods

Fungal collections

Fungal specimens were collected from Guangdong Province, China. Tissue blocks were removed from the inner part of fresh basidiomata for DNA analyses. The specimens were dried with an electric air-ventilation drier and deposited in either the Fungal Herbarium of Shenyang Agricultural University, Shenyang, China (SYAU), or the Mycological Herbarium of Guangdong Institute of Microbiology, Guangzhou, China (GDGM).

Morphological observations

The macroscopic description is based on the fresh specimens. The names of colors are based on Ridgway (1912). Microscopic observations were made on sections of dried specimens, using a 5% KOH solution and in Melzer's reagent, using a light microscope. The Q-value (quotient of length/width) for each spore (20 spores for each specimen) was calculated, and the mean values were used in the descriptions.

For observation of the surface of spores, the gills were coated with gold. The spores were examined with a scanning electron microscope (SEM; Zeiss & Ultra Plus, Germany).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the fresh blocks of tissue of the specimens using the Plant DNA Extraction Kit (Sunbiotech Co., Ltd, Beijing). The raw DNA extracts were used as templates for PCR. Amplification primers were ITS5/ITS4 (White et al.

1990) for the ITS1+5.8S+ITS2 region and LROR/LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) for the 5¢ end of 28S rRNA gene. Both reaction mixtures and PCR conditions followed those in Yu et al. (2014). The PCR products were checked on a 1% agarose gel stained with ethidium bromide. The DNA bands were visualized on a UV transilluminator and documented digitally using the BIO-RAD ChemiDoc XRS imaging system. Sequencing was performed on an ABI Prism® 3730 Genetic Analyzer (PE Applied Biosystems, USA).

DNA sequence analyses

The BLAST database (Altschul et al. 1997) was searched using the ITS and nrLSU sequences from the type collection (SYAU-FUNGI-009) as the queries (23 May 2016). In order to confirm the taxonomic position of the new species, the sequences of the Tricholomatoid clade (Matheny et al. 2006) were also retrieved from GenBank. Nucleotide sequences amplified from the collections of the new species were aligned with 44 ITS and 51 LSU sequences retrieved from GenBank using BioEdit 5.0.6 (Hall 1999) and Clustal X (Thompson et al. 1997). Based on the results of Matheny et al. (2006), the sequences of *Agaricus bisporus* (J.E. Lange) Imbach (DQ404388 for ITS; AY635775 for nrLSU) were downloaded and included for rooting purposes.

A few dozen bases at both ends of the sequences were excluded from the analyses because of the uncertainty in the base cells of the sequences. Finally, the data matrices with 689 bp for ITS and 955 bp for nrLSU were produced. Nodal bootstrap support (BS) was assessed with nonparametric bootstrapping using 1000 replicates. Bayesian analysis was conducted using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). The best-fitting sequence evolution model was chosen using MrModelTest v. 2.2 (Nylander 2004). Bayesian analyses of the ITS and nrLSU regions were run for 2 000 000 generations, all under the GTR model, with four chains, and trees sampled every 100 generations. The average split frequencies were checked to determine optimal convergence of the chains below 0.01 after each generation. The first 25% (1250 trees) of the sample trees was designated as burn-in, with the remaining samples retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree for Posterior Probabilities (PP). Alignments have been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19825 and S19826).

Results

Four ITS sequences and four nrLSU sequences were obtained from fresh basidiomata. The average standard deviation of split frequencies in the Bayesian analysis was less than 0.01 after 2,000,000 generations. Both trees were rooted with *Agaricus bisporus* (Figs. 1, 2). The ITS and nrLSU sequence analyses both placed the genus *Pseudobaeospora* in a monophyletic group (PP = 1) sister to *Callistosporium graminicolor* Lennox (*Tricholomataceae*). The new species, *P. lilacina*, grouped with the other *Pseudobaeospora* species (*P. deckeri* C.F. Schwarz, *P. pyrifera* Bas & L.G. Krieglst., *P. wipapatiae* Desjardin et al.) but formed a separate branch with strong support (PP = 1).

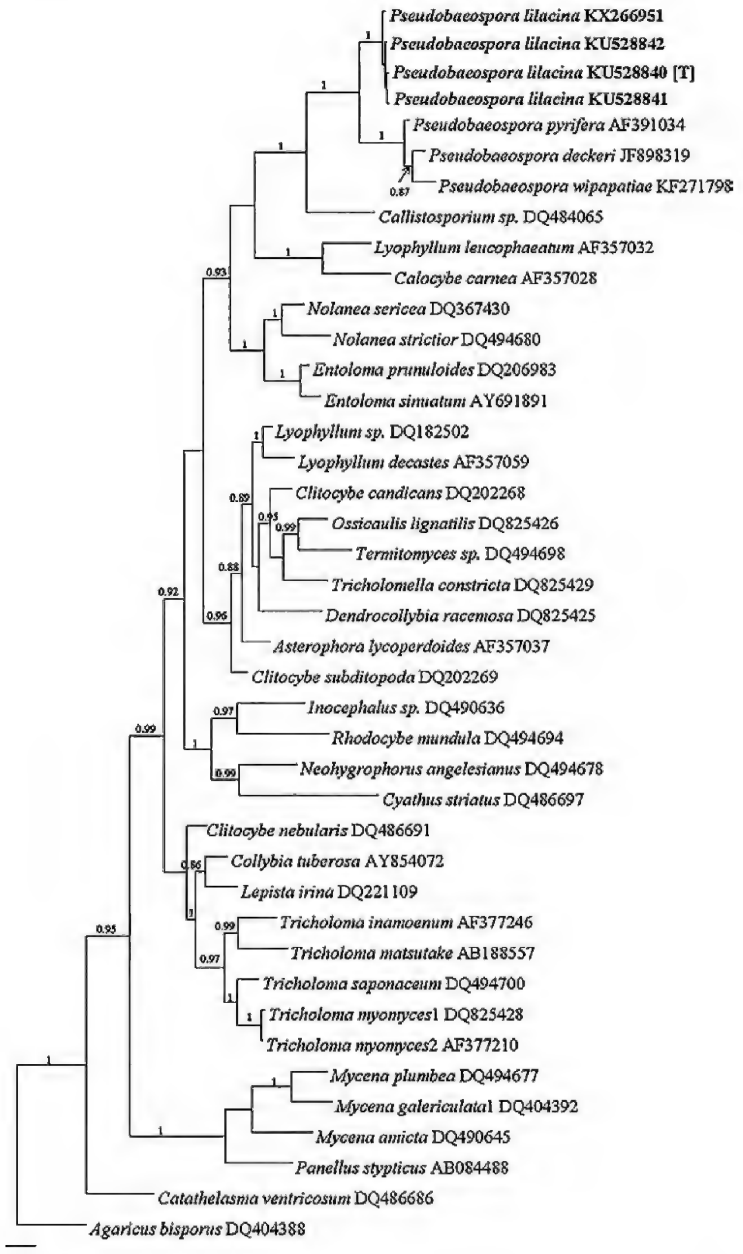

FIG. 1. Fifty percent majority-rule Bayesian cladogram of *Pseudobaeospora* and related taxa based on ITS sequence analysis. Node support is indicated by Bayesian posterior probabilities >0.5 on branches. *Pseudobaeospora lilacina* sequences are presented in boldface.

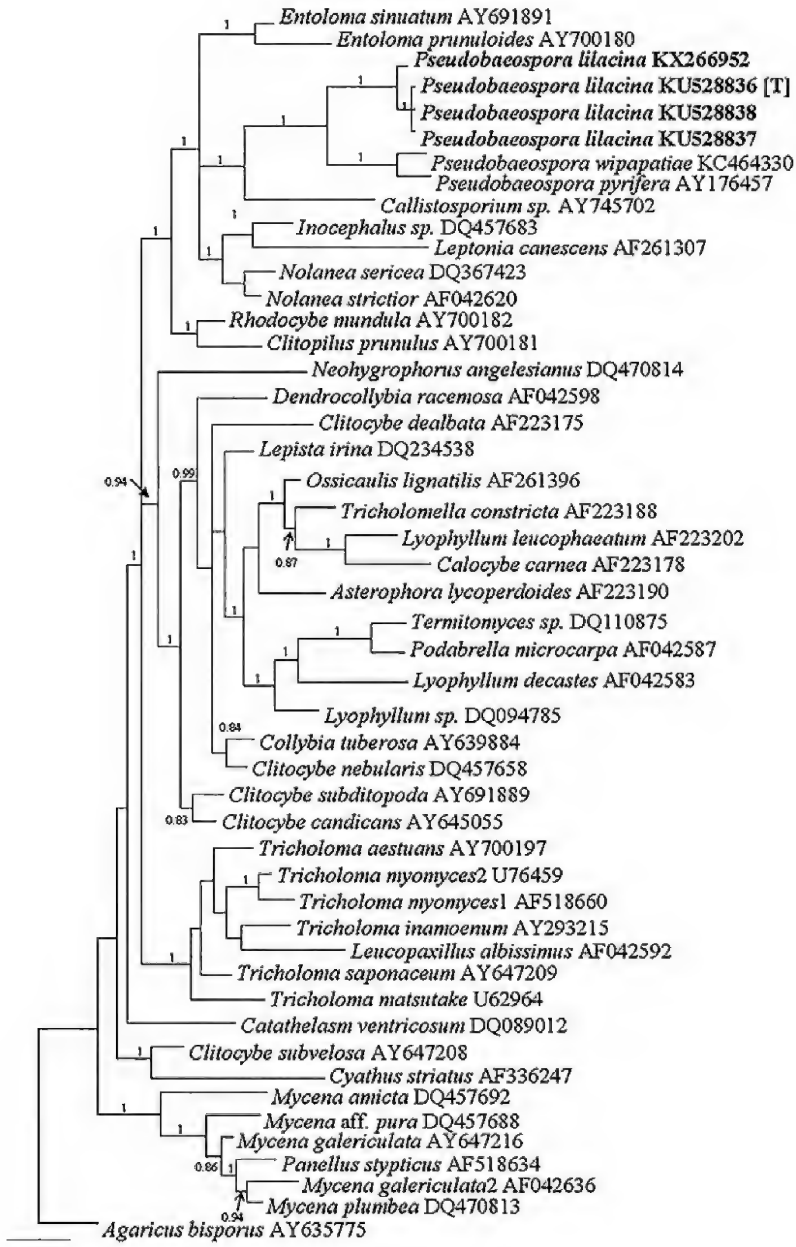

FIG. 2. Fifty percent majority-rule Bayesian cladogram of *Pseudobaeospora* and related taxa based on nrLSU sequence analysis. Node support is indicated by Bayesian posterior probabilities >0.5 on branches. *Pseudobaeospora lilacina* sequences are presented in boldface.

Taxonomy

Pseudobaeospora lilacina X.D. Yu, Ming Zhang & S.Y. Wu, **sp. nov.** FIG. 3

MycoBank MB 815567

Differs from other species in the Frieslandica group by its lilac pileus, stubbly stipe, and ellipsoid spores.

Type: China. Guangdong Province, Guangzhou City, Baiyun Mountain, on grass, 28 May 2013, Zhang M 3459 (**Holotype**, SYAU FUNGI-009; GenBank KU528840, KU528836).

Etymology: The epithet *lilacina* refers to the lilac pileus color.

Pileus 1–3 cm diam, plano-convex to almost flat, dry; surface pale mauve, colonial buff when old, slightly squamulose, darker at center, hygrophanous; margin flexuous to involute. Context 0.1–0.2 cm thick at stipe, thin at margin, whitish to tinged with pileus' color. Lamellae adnate to uncinate, arched, ≤3 mm broad, unequal, distant, with lamellulae of 2–4 lengths but not anastomosing, pale mauve to mauve, undulate and thin at edge. Stipe 2–3 × 0.2–0.5 cm, cylindrical, somewhat expanded at base, sometimes pliant, pale Congo pink, fibrous, striate, central, pruinose to flocculose overall, bruising indigo-bluish. Odor inconspicuous.

Basidiospores 2.5–3.5(–4.5) × 3–5(–6.5) µm, Q = 1.38, broadly ellipsoid to ellipsoid, hyaline, thick-walled, dextrinoid, minutely rough and with a distinct hilar appendage under scanning electron microscope. Basidia 14–25 × 3.0–5.8 µm, 4-spored and 2-spored. Hymenium hyphae 3.5–7 µm diam, hyaline, thin-walled, non-amyloid; cystidia absent. Hymenophoral trama 100–180 µm wide, regular, hyphae 2.8–7.3 µm, thin-walled. Pileipellis a trichoderm of ascending cylindrical hyphae, 6–10 µm diam, thin-walled, hyaline; suprapellis composed of regular parallel hyphae, 4.2–8.3 µm diam, thin-walled, hyaline, color unchanging in 5% KOH. Clamp connections present in all parts of the basidiocarp.

Habit saprophytic, solitary on naked soil or on grass.

Additional specimens examined: **CHINA. Guangdong Province, Zhaoqing City,** Fengkai County, Heishiding Nature Reserve, 3 June 2013, Yu XD 5546 (SYAU FUNGI-011; GenBank KU528842, KU528838); **Guangzhou City,** Baiyun Mountain, 28 May 2013, Yu XD 3450 (SYAU FUNGI-010; GenBank KU528841, KU528837); 22 June 2008, Li TH, Chen XL, Li Y (GDGM25609; GenBank KX266951, KX266952); 22 May 2011, Xu J (GDGM44799); 25 May 2011, Xu J, Chen XL, Zhang M (GDGM28870); 27 May 2011, Xu J, Qiu CS, Zhang M (GDGM28919).

Discussion

Pseudobaeospora lilacina represents the first report of *Pseudobaeospora* in China. Based on morphology, the new species should be placed in the

FIG. 3. *Pseudobaeospora lilacina* (holotype, SYAU FUNGI-009). A, B: Macroscopic habit. C, D: Surface of spores. Scale bars: A, B = 1 cm; C = 1 µm; D = 2 µm.

Frieslandica group: colored basidiocarp, presence of clamp connections, and non-hymenidermoid pileipellis (Bas 2003). However, its pale mauve pileus and stubbly stipe easily distinguish *P. lilacina* from the other Frieslandica species. Although most of the other species have purple tones in the pileus, their colors are conspicuously deeper (Bas 2003) than those found in *P. lilacina*. *Pseudobaeospora subglobispora* Bas from Asia shares a similar pileus color, but is distinguished from *P. lilacina* by its shorter (0.4–1 cm) stipe and obvious cheilocystidia. Three additional Asian species—*P. citrina* Rawla, *P. lavendulamellata* Arnolds et al., *P. oligophylla*—are easily distinguished from *P. lilacina*: *P. citrina* by a greenish yellow pileus (Bas 2003), *P. lavendulamellata* by a dull violet pileus (Arnolds et al. 2004), and *P. oligophylla* by a dark violaceous pileus (Bas 2003). Furthermore, in *P. oligophylla* there are no clamp connections in the basidiocarp hyphae (Bas 2003), and *P. lavendulamellata* has obvious cheilocystidia and its pileipellis discolors in 5% KOH (Arnolds et al. 2004).

We found only three *Pseudobaeospora* species—*P. deckeri*, *P. pyrifera*, *P. wipapatiae*—with available GenBank sequences, indicating the deficiency of molecular research on the genus. However, our molecular analyses provide some clues as to the taxonomic position of *Pseudobaeospora*. Both ITS and nrLSU sequence analyses (Figs 1, 2) placed the *Pseudobaeospora* species and *Callistosporium graminicolor* into one clade supported by strong Posterior Probabilities (PP = 1), with the clade clustering with *Lyophyllaceae* or *Entolomataceae*. Our result is inconsistent with the previous nrLSU sequence analysis by Desjardin et al. (2014), which placed *Pseudobaeospora* in *Tricholomataceae*. The reason for the inconsistency is not clear but may be explained by the low number of sequences available or taxa selected for analysis. Even so, our result supports the observation by Vellinga (2004) that *Pseudobaeospora* does not belong in *Agaricaceae*.

Pseudobaeospora will require more sampling and molecular analyses before its taxonomic position can be established.

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Gymnopilus dilepis, a new record in Thailand

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Abstract—Specimens collected in Thailand have been identified as *Gymnopilus dilepis*, based on morphology and ITS molecular analysis. A description and illustration are provided. This is the first record of this fungus from Thailand.

Key words—*Agaricales*, *Cortinariaceae*, gill fungus, taxonomy

Introduction

Karsten (1879) erected the genus *Gymnopilus,* with *Agaricus liquiritiae* Pers. [≡ *G. liquiritiae* (Pers.) P. Karst.] as the type species. *Gymnopilus* has been treated as a member of *Cortinariaceae*, *Agaricales* (Singer 1986). This genus of saprotrophic fungi is distributed worldwide in temperate and tropical regions, usually occurring on dead wood (Singer 1986; Guzmán-Dávalos 2003), and approximately 200 species have been reported (Kirk et al. 2008). Four *Gymnopilus* species—*G. aeruginosus* (Peck) Singer, *G. junonius* (Fr.) P.D. Orton, *G. penetrans* (Fr.) Murrill, and *G. punctifolius* (Peck) Singer—have been reported from Thailand (Chandrasrikul et al. 2011). During a taxonomic survey of macrofungi collected in northern Thailand, we collected specimens that corresponded to the description of *G. dilepis*, a species previously reported from Australia, India, Indonesia, Japan, Malaysia, Papua New Guinea, Sri Lanka, and the United Kingdom (Pegler 1986, Treu 1998, Guzmán-Dávalos

2003, Guzmán-Dávalos et al. 2003, Thomas et al. 2003, Rees et al. 2004, Kasuya et al. 2016). Here we describe and illustrate the morphological characters of the Thai material and provide results from ITS sequence analysis.

Materials & methods

Morphology studies

Basidiocarps were collected from Lampang Province, Thailand, in 2015 and wrapped in aluminum foil or kept in plastic specimen boxes for transport to the laboratory. Notes on macromorphological features and photographs were obtained within 24 h of collection. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C; sections of dried material were mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent for microscopical examination; at least 50 measurements were made of each structure. The collections are deposited in the herbarium of the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of two specimens was extracted from fresh tissue using a Genomic DNA Extraction Mini Kit (Favorgen, Taiwan). The internal transcribed spacer (ITS) region of the ribosomal RNA gene was amplified by the polymerase chain reaction (PCR) with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and a PCR Clean-up Kit (Macherey-Nagel, Germany), following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was carried out by 1ST Base Company (Kembangan, Malaysia) using the ITS4 and ITS5 primers. Sequences were used to query the GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

Results

Taxonomic description

Gymnopilus dilepis (Berk. & Broome) Singer, Lilloa 22: 560 (1951). Fig. 1

Pileus 20–50 mm in diameter, convex to plano-convex, surface light orange (6A5) towards the center, pale orange (5A3) to light orange (5A4) towards the margin, squamulose; squamules ruby (12D8) to violet brown (10F8), erect and comparatively denser at the center, sparse and appressed in the middle, sparse or almost absent towards the margin; margin decurved to plane. Context concolorous with the pileus surface. Lamellae adnate to subdecurrent, light orange (5A5) to orange (5A6). Stipe 25–60 × 3–9 mm, central, almost equal or slightly tapering basally, surface pale orange (5A3), becoming dark brown

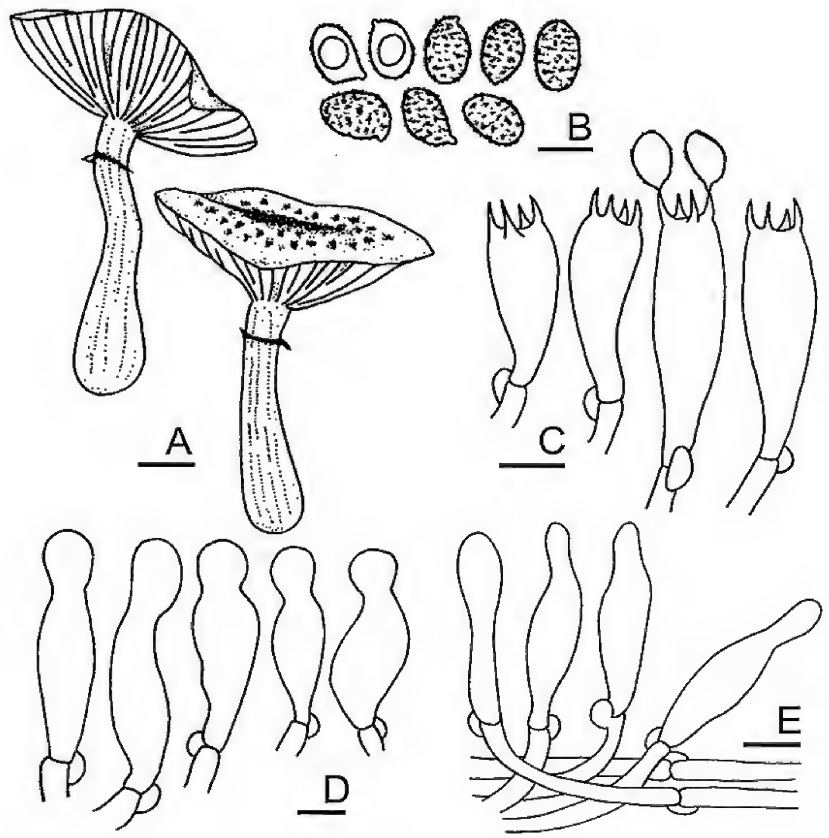

Fig. 1. *Gymnopilus dilepis* (SDBR-CMU-NK0116).
A: basidiocarps; B: basidiospores; C: basidia and basidiospores; D: cheilocystidia; E: caulocystidia.
Scale bars: A = 10 mm, B–D = 5 μm, E = 10 μm.

(6F6) towards base. Veil present. Odor slight. Pileus and stipe surface darkened to brownish orange (6C8) with aqueous KOH. Spore print light brown (6D8).

Basidiospores 6–7.5 × 4.8–6 μm, Q = 1.23–1.5, broadly elliptical to ellipsoid, with obtuse apex, slightly thickened walls, verrucose, warts medium to large, dextrinoid, orange-brown in KOH. Basidia 20–27.5 × 5–8.5 μm, clavate to cylindric-clavate, hyaline, 4-spored, with basal clamp connection, sterigmata ≤5.5 μm long. Pleurocystidia absent. Pseudocystidia 20–27 × 7–8.5 μm, clavate-rostrate or subfusiform, thin-walled, with granulose to homogeneous orange-brown contents. Cheilocystidia 20–30.2 × 8–12 μm, apex 2.2–4.5 μm diam.,

utriform, ranging from clavate with a wide rostrum to lageniform with a short neck and a non-capitate or subcapitate apex, thin-walled, with basal clamp connection, hyaline to pale yellow. Hymenophoral trama subregular, hyphae 2–20 µm diam., thin-walled, hyaline to pale yellow. Subhymenium inflated-ramose. Pileal trama radial, hyphae 2–20.5 µm diam., thin-walled, hyaline to pale yellow. Pileipellis a cutis, hyphae 3–15 µm diam., thin-walled, coarsely encrusted with brown pigment. Stipe trama composed of hyphae 2–25 µm diam., parallel, thin-walled, pale yellow. Stipitipellis a cutis, hyphae 2–12 µm diam., thin-walled with pale yellow to brown wall pigment. Caulocystidia 18.2–65 × 5.5–14.5 µm, cylindrical, clavate, narrowly utriform, with obtuse or subcapitate apex, hyaline, some with granulose, pale yellow or orange-brown content, thin-walled, in tufts at the stipe apex. Clamp connections present on all hyphae.

Specimens examined—**THAILAND**, **Lampang Province**, Mae Moh District, 18°40′52″N 98°52′10″E, elevation 550 m, on dead wood in a deciduous forest, 8 November 2015, Suwannarach N & Lumyong S (SDBR-CMU-NK0116; GenBank KX639496); Kumla J & Suwannarach N (SDBR-CMU-JK0142; GenBank KX639497).

Molecular analysis

The ITS sequences of specimens SDBR-CMU-NK0116 (644 bp) and SDBR-CMU-JK0142 (638 bp) were deposited in GenBank. All ITS sequences obtained in this study showed 100% similarity with *G. dilepis* KT368680 and KT368682, from Japan.

Discussion

Both specimens collected in northern Thailand were initially identified as *G. dilepis* based on descriptions by Guzmán-Dávalos (2003) and Thomas et al. (2003). This species is easily distinguished from *G. penetrans*, which produces a gray-brown to dark brown pileus with a white to grayish-white tomentose-arachnoid velum covering the whole surface (Holec 2005). The smaller basidiospores (4.0–6.0 × 3.5–5.0 µm) of *G. punctifolius* and the longer basidiospores (8.0–10.5 × 5.5–7.2 µm) of *G. junonius* clearly distinguish these two species from *G. dilepis* (Singer 1951; Holec 2005).

Gymnopilus dilepis is closely related to *G. purpuratus* (Cooke & Massee) Singer and *G. norfolkensis* B.J. Rees & Lepp, from which it is distinguished by differences in spore size: basidiospores of *G. purpuratus* differs are longer (7.5–8.7 × 4.8–5.7 µm; Rees et al. 2004) while those of *G. norfolkensis* are narrower (6.4–7.2 × 4.0–5.2 µm; Rees and Lepp 2000). *Gymnopilus dilepis* also resembles *G. lepidotus* Hesler, but the two species differ in the form of

the cheilocystidia; additionally *G. lepidotus* is known only from Mexico and the United States (Hesler 1969, Guzmán-Dávalos 2003, Guzmán-Dávalos et al. 2003).

Our molecular analysis also confirmed the two Thai specimens as representing *G. dilepis*. The combination of morphological and molecular characters confirms *G. dilepis* as a new record for Thailand.

Acknowledgements

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A reassessment of *Hourangia cheoi* from Yunnan, China

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Abstract—A recent collection of *Hourangia cheoi* (*Boletaceae*, *Boletales*) from near the type locality in Yunnan Province, southwestern China, is described in detail and illustrated, and its nrITS and nrLSU rDNA sequences are included in molecular phylogenetic analyses. Comparisons with closely related xerocomoid taxa are also provided.

Key words—biogeography, ectomycorrhizal fungi, taxonomy, *Xerocomus*

Introduction

During repeated mycological field expeditions carried out by the first author during 2011–13 in central, southwestern, and southeastern China (Beijing, Shaanxi, Yunnan, and Guangdong Provinces), several new or noteworthy fungal species were recorded and documented (Gelardi et al. 2012, 2013, 2014, 2015a,b; Vizzini et al. 2012, 2014; Battistin et al. 2014, Gelardi 2014, Picillo & Gelardi 2014). Amongst the several bolete species found in the surveyed areas, a single specimen with a xerocomoid habit was collected near Dali, Yunnan, and later identified from morphological and molecular analyses as *Hourangia cheoi*.

Chiu (1948) originally described the species in the genus *Boletus* Fr. In the same paper the Chinese author described *Boletus punctilifer;* both species were subsequently illustrated (Chiu 1957) and later combined in *Xerocomus* Quél. (Tai 1979). However, Zhu et al. (2015) reduced *B. punctilifer* to synonymy under *B. cheoi* after examining numerous specimens, including the holotypes of both names.

As a result of intensive research on Chinese xerocomoid boletes, Zhu et al. (2015) erected a new genus *Hourangia* based on *Boletus cheoi,* including three other species originally described as *B. microcarpus* Corner, *B. nigropunctatus* W.F. Chiu, and *Phylloporus pumilus* M.A. Neves & Halling. *Hourangia* is related to *Xerocomus* s.str. and sister to *Phylloporus* Quél. (Wu et al. 2014, Zhu et al. 2015, Henkel et al. 2016), and despite the phylogenetic and morphological affinities with these genera, it is readily distinguished by a finely squamulose pileus surface, tubes that are three to seven times longer than the thickness of pileus context, whitish basal mycelium, context turning blue then reddish and finally fading to blackish–brown on exposure, a bacillate spore ornamentation (visible only with a scanning electron microscope), a "*Phylloporus*–type" hymenophoral trama, ectomycorrhizal association with *Fagaceae, Pinaceae,* and *Dipterocarpaceae,* and its eastern and southeastern Asian distribution (Zhu et al. 2015; pers. obs.).

The aim of the present contribution is to provide new insights and iconography on *Hourangia cheoi.*

Materials & methods

Collection site and sampling

The specimen was collected in the surroundings of Dali, Yunnan Province, China, and is deposited in ZT (Thiers 2017) and in the personal herbarium of Matteo Gelardi (MG). Author citations follow Index Fungorum (www.indexfungorum.org/authorsoffungalnames.htm).

Morphological studies

Macroscopic descriptions, habitat notations, and associated plant communities were based upon detailed field notes from fresh basidiomes. Color terms in capital letters (e.g. Myrtle Green, pl. VIII) are from Ridgway (1912). The specimen was photographed in habitat using a Nikon D3100 camera. Micro–morphologic features were observed from dried material; sections were rehydrated in water, 5% potassium hydroxide (KOH), or ammoniacal Congo Red. Structures and anatomical features were observed and measured in Congo Red; color and pigments were observed in KOH. Measurements were determined at 1000× with a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Basidiospores were measured from the mature hymenophore; dimensions are given as (minimum–) average ± standard deviation (–maximum), Q = length/width (L/W) ratio with the extreme values in parentheses, Qm = average quotient (L/W ratio) ± standard deviation, and average spore volume was approximated as a rotation ellipsoid ($V = (\pi.L.W^2)/6$ ± standard deviation). The notation [n/m/p] indicates that measurements were made on "n" randomly selected basidiospores from "m" basidiomes of "p" collections. The width of each basidium was measured at the widest part, and the length was measured from

the apex (sterigmata excluded) to the basal septum. Metachromatic, cyanophilic, and iodine reactions were tested by staining the basidiospores in Brilliant Cresyl blue, Cotton blue and Melzer's reagent, respectively. Line drawings of microstructures were made freehand based on photomicrographs of rehydrated material.

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from the dry specimen (MG484) with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. PCR primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) for the nrITS region, LR0R, LR1, LR5 and LR7 (Vilgalys & Hester 1990, Cubeta et al. 1991, Van Tuinen et al. 1998) for the nrLSU region, were employed for PCR amplification and sequencing purposes. The PCR protocol comprised a hot start at 95°C (5 min), followed by 35 cycles of 94°C (45 s), 54°C (30 s), and 72°C (45 s), and a final 72°C step (10 min). PCR products were checked in 1% agarose gels before purification and sequencing. Chromatograms were searched for putative sequencing reading errors. The sequences are deposited in GenBank and their accession numbers are included in Figs 1, 2.

Sequence alignment, dataset assembly and phylogenetic analyses

The sequences obtained in this study were checked and assembled using Geneious v. 5.3 (Drummond et al. 2010) and compared to those available in GenBank using the Blastn algorithm. Based on the BLASTn results (with sequences selected based on greatest similarity) and the phylogenetic analysis by Zhu et al. (2015), sequences were retrieved from GenBank and UNITE (http://unite.ut.ee/) databases for the comparative phylogenetic analysis. Alignments were generated for each single LSU and ITS dataset using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The two alignments were then imported into MEGA v. 6.0 (Tamura et al. 2013) for manual adjustment. The best-fit models were estimated by the Bayesian Information Criterion (BIC) using jModelTest v. 2.1.7 (Darriba et al. 2012) to provide a substitution model for each single alignment. GTR+G models were chosen for both the ITS and LSU alignments.

Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum likelihood (ML) approaches. *Phylloporus rhodoxanthus* (JQ003654), *Xerocomus ferrugineus* (JQ003657) and *X. subtomentosus* (KC215201) were chosen as the outgroup taxa in the ITS analysis (Fig. 1); and *Phylloporus imbricatus* (KF112398), *P. luxiensis* (KF112490), *P. pelletieri* (AF456818), *P. rubrosquamosus* (KF112391), *"Xerocomus perplexus"* (JQ003702), and *X. subtomentosus* (KC215222) in the LSU analysis (Fig. 2), following Zhu et al. (2015). Bayesian and ML inferences were performed online using the CIPRES Science Gateway website (Miller et al. 2010) and both methods were implemented as single software usage. BI phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes v. 3.2.2 (Ronquist et al. 2012). Four incrementally heated simultaneous MCMC were run over 10 M generations. Trees were sampled every 1000 generations resulting in an overall sampling of 10,001 trees. The first 2500 trees (25%) were discarded as burn-

in. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. ML estimation was performed through RAxML v. 7.0.4 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the 'f a' option of RAxML and '-x 12345' as a random seed to invoke the novel rapid bootstrapping algorithm. Only BPP values >0.75 and/or MLB values >50% are reported in the resulting trees.

Results

Both Bayesian and Maximum Likelihood analyses produced the same topology; only the BI trees with both BPP and MLB values are shown (Figs 1, 2).

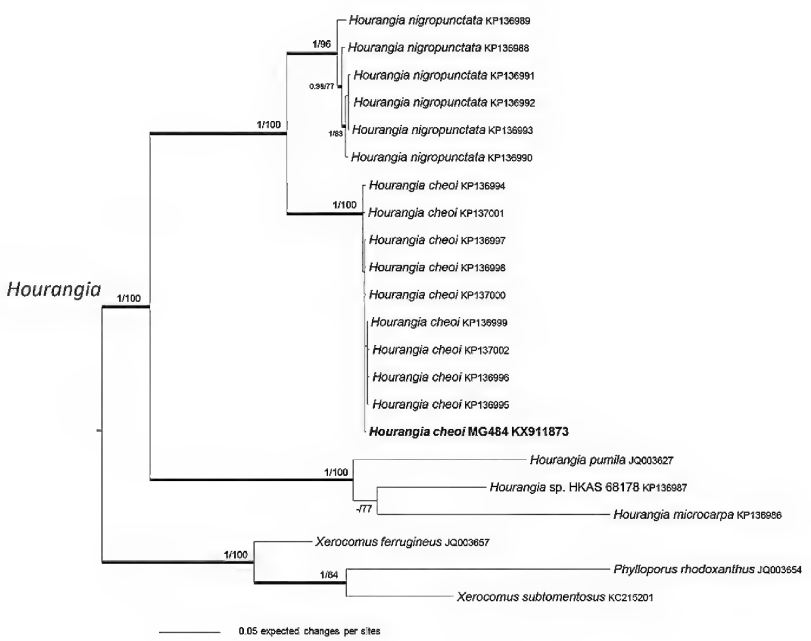

Fig. 1. Bayesian phylogenetic analysis based on the ITS sequences of *Hourangia* species and related taxa. Bayesian posterior probabilities (BPP; in bold) ≥0.75 and Maximum Likelihood bootstraps (MLB) ≥50% are shown on the branches. Thickened branches indicate BPP ≥0.95 and MLB ≥70%. The GenBank/UNITE number is given for each sequenced taxon. The new *H. cheoi* sequence is in bold.

The ITS data matrix comprised a total of 22 sequences (including 21 from GenBank); the alignment comprised 1160 characters and contained 526 (45.3%) variable sites, of which 284 (24.5%) were parsimony informative. The LSU matrix consisted of 34 sequences (including 33 from GenBank); the alignment comprised 830 characters and contained 144 (17.3%) variable sites, of which 110 (13.2%) were parsimony informative.

Both in the ITS and LSU analyses, our collection and the *Hourangia cheoi* sequences retrieved from GenBank form a strongly supported clade (BPP = 1 and MLB = 100 in the ITS analysis; BPP = 0.95 and MLB = 95 in the LSU analysis).

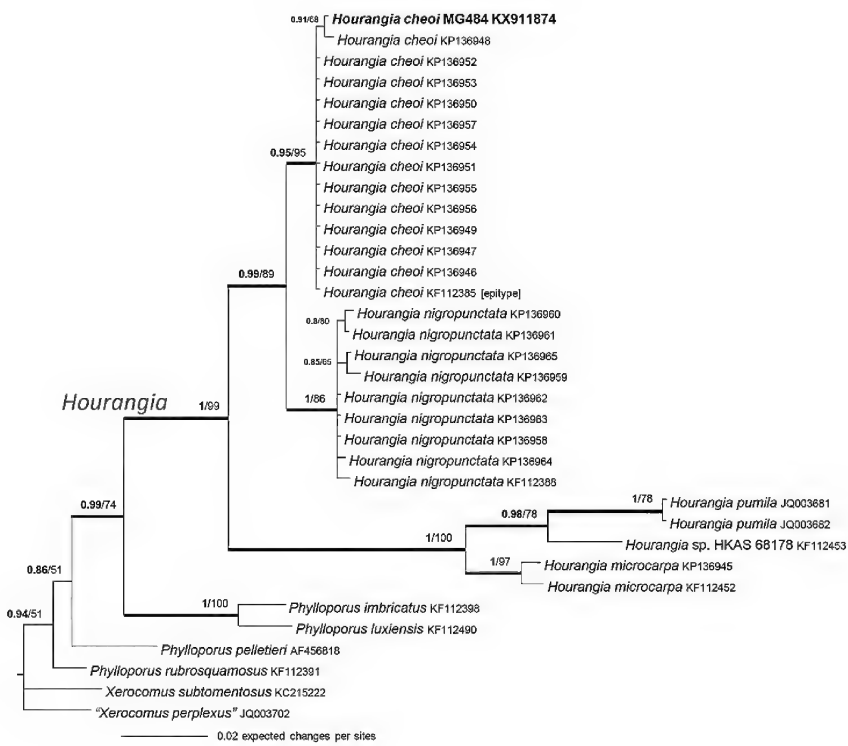

Fig. 2. Bayesian phylogenetic analysis based on the LSU sequences of *Hourangia* species and related taxa. Bayesian posterior probabilities (BPP; in bold) ≥0.75 and Maximum Likelihood bootstraps (MLB) ≥50% are shown on the branches. Thickened branches indicate BPP ≥0.95 and MLB ≥70%. The GenBank number is given for each sequenced taxon. The new *H. cheoi* sequence is in bold.

Taxonomy

Hourangia cheoi (W.F. Chiu) Xue T. Zhu & Zhu L. Yang, Mycol. Progr. 14: 37 [4 of 10] (2015). FIGS 3, 4

MYCOBANK MB 810696

≡ *Boletus cheoi* W.F. Chiu, Mycologia 40(2): 215 (1948).

≡ *Xerocomus cheoi* (W.F. Chiu) F.L. Tai, Syll. Fung. Sinicorum: 813 (1979).

= *Boletus punctilifer* W.F. Chiu, Mycologia 40(2): 216 (1948).

≡ *Xerocomus punctilifer* (W.F. Chiu) F.L. Tai, Syll. Fung. Sinicorum: 814 (1979).

BASIDIOMES medium-small. PILEUS 4.3 cm diam., convex-pulvinate, regularly shaped, moderately fleshy, soft; margin straight to faintly wavy, curved downwards, not or only slightly extending beyond the tubes; surface matte, dry, very finely squamulose, not cracked; cuticle evenly dark ochraceous (Buckthorn Brown, pl. XV; Sayal Brown, pl. XXIX); slowly darkening to dull brownish-black (Warm Sepia, pl. XXIX) on handling or when injured, especially at the edge of squamules; subcuticular layer whitish (White, pl. LIII). TUBES somewhat broad and decidedly longer than the thickness of pileus context (≤1.0 cm long), adnate, olive yellow (Javel Green, pl. V), turning blue (Myrtle Green, pl. VIII) when cut. PORES forming a slightly convex or ascendant surface, simple, not radially arranged, prominently wide and angular (≤1 mm in diam.), concolorous with the tubes and turning blue (Myrtle Green, pl. VIII) on bruising or when injured and finally fading to brownish (Snuff Brown, pl. XXIX), without innate rusty brown stains at the orifice. STIPE 5.3 × 0.6 cm, slightly longer than pileus diameter, central, solid, firm, dry, faintly curved, cylindrical but slightly swollen at the base, ending with a short taproot at the very base; surface finely longitudinally fibrillose, devoid of reticulum or any other kind of ornamentation, veils absent; beige-ochraceous (Cream Buff, pl. XXX) throughout but paler (Cartridge Buff, pl. XXX) towards the base, unchangeable when pressed; basal mycelium whitish (White, pl. LIII), rhizomorphs brownish (Dresden Brown, pl. XV). CONTEXT soft textured and thin in the pileus (≤0.4 cm thick in the central zone), a little more fibrous in the stipe, whitish (White, pl. LIII) throughout but pale yellow (Martius Yellow, pl. IV) above the hymenophore; turning light blue (Pale Methyl Blue; Light Cerulean Blue, pl. VIII; Sky Blue, pl. XX) in the pileus and stipe/pileus connecting zone when exposed to air but changing within twenty seconds to bright flesh pink (Buff Pink, Congo Pink, pl. XXVIII) and eventually fading to grayish (Light Mouse Gray, pl. LI) in patches, elsewhere in the stipe directly turning to pinkish–red (Congo Pink, Japan Rose, Terra Cotta, pl. XXVIII) after cutting, especially towards the base, then fading to dirty grayish (Mouse

Fig. 3. *Hourangia cheoi* (MG484).
Basidiome in habitat. Scale bar = 2 cm. Photo by M. Gelardi.

Gray, pl. LI) or grayish–black (Blackish Mouse Gray, Sooty Black, pl. LI); subhymenophoral layer yellowish (Martius Yellow, pl. IV); exsiccate brownish (Dresden Brown, pl. XV). Smell hardly perceptible, fungoid. Taste mild. Spore print not obtained.

Basidiospores [40/1/1] (9.8–)11.2 ± 0.8(–13.1) × (4.0–)4.6 ± 0.2(–5.0) µm, Q = (2.17–)2.22–2.72(–2.75), Qm = 2.44 ± 0.14, V = 126 ± 20 µm^3, inequilateral, ellipsoid-fusiform to fusiform in side view, ellipsoid-fusiform in face view, smooth under the light microscope, with a prominent apiculus and shallow suprahilar depression, apex rounded, moderately thick-walled (0.5–0.7 µm), straw yellow to bright yellow colored in KOH, having one or (less frequently) two large oil droplets when mature, rarely pluri-guttulate, inamyloid to faintly dextrinoid, cyanophilic and with an orthochromatic reaction; no anomalous spores observed. Basidia 28–37(–40) × 9–11 µm (n = 10), cylindrical-clavate to clavate, moderately thick-walled (0.5–0.8 µm), predominantly 4-spored but

also 2- or (sporadically) 3-spored, usually bearing relatively long sterigmata (2–7 µm), hyaline to very pale yellowish and containing straw-yellow oil guttules in KOH, bright yellow to orange-reddish (barely dextrinoid) in Melzer's, without basal clamps; basidioles subcylindrical to faintly clavate, similar in size to basidia. Cheilocystidia (40–)51–76(–82) × 6–11 µm (n = 17), very common, moderately slender, projecting, straight to sometimes flexuous, cylindrical to cylindrical-fusiform, rarely subclavate and occasionally subcapitate or mucronate, with rounded tip, smooth, moderately thick-walled (0.8–1.0 µm), hyaline or with a very pale yellowish pigment in KOH, bright yellow (inamyloid) in Melzer's and with an orthochromatic reaction, without epiparietal encrustations. Pleurocystidia (62–)67–110(–113) × 7–23 µm (n = 21), decidedly frequent, color and chemical reactions similar to cheilocystidia but slightly different in shape and distinctly longer, straight, slender, cylindrical-fusiform or fusiform to ventricose-fusiform, less frequently lageniform, sometimes with a narrow and long neck, apex rounded. Pseudocystidia not recorded. Pileipellis a trichoderm or palisadoderm consisting of subparallel to loosely interwoven, elongated, cylindrical, rarely branched hyphae tending to remain erect in the outermost layer and not embedded in gelatinous matter; terminal elements usually long, slender and markedly swollen (cylindrocytes), cylindrical to sausage-shaped, less frequently cystidioid, subclavate or peanut-shaped, acorn-shaped or bullet-shaped, apex rounded-obtuse, (36–)41–88(–103) × (12–)15–29(–33) µm (n = 26), intermixed with scattered longer, filamentous and sinuous cells (up to 127 × 13 µm), moderately thick-walled (≤1 µm); pileipellis elements often appear dissociated and easily separable from one another, hyaline to (more often) yellowish in KOH, bright yellow to orange-reddish (weakly dextrinoid) in Melzer's, smooth; subterminal elements similar in shape, size, color and chemical reactions to terminal elements. Stipitipellis a texture of slender, subparallel to parallel and longitudinally running, smooth walled, adpressed hyphae, 4–19 µm wide, hyaline in KOH; the stipe apex covered by tufts of caulohymenium consisting of sterile caulobasidioles, very sparse 2-/3-spored fertile caulobasidia similar in shape, size and color to hymenial basidia; Caulocystidia abundant, long, subcylindrical to fusiform, ventricose-fusiform or lageniform, projecting, (33–)44–82(–92) × 7–10(–15) µm (n = 14), apex rounded, wall ≤1 µm thick, hyaline to yellowish in KOH. Lateral stipe stratum under the caulohymenium absent. Stipe trama hyphae longitudinal, densely arranged, subparallel to moderately interwoven, filamentous, smooth to rarely encrusted by a very subtle granular, brownish pigment, inamyloid to barely dextrinoid, 4–20 µm diam. Hymenophoral

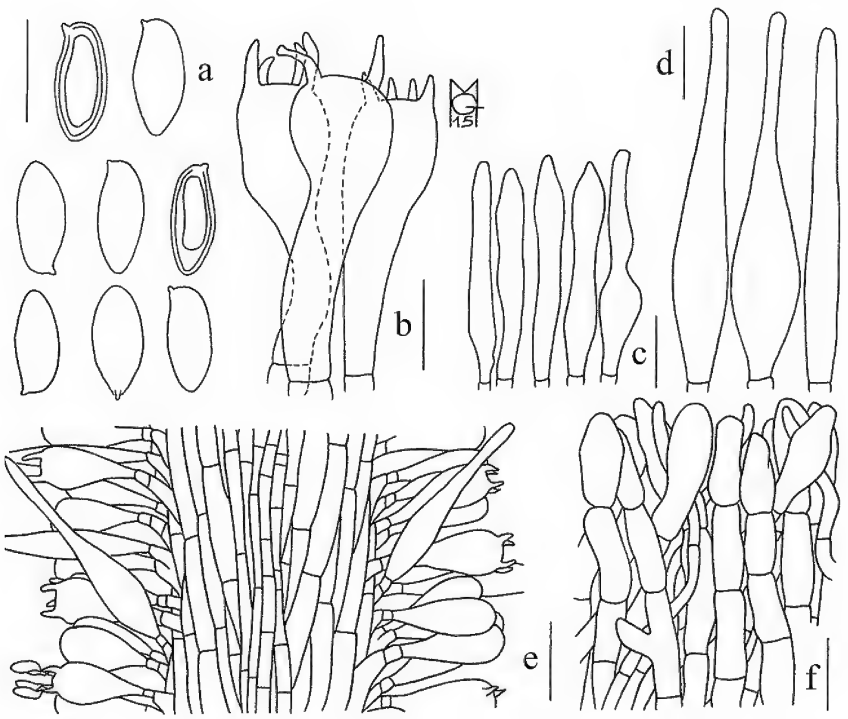

FIG. 4. *Hourangia cheoi* (MG484). a. Basidiospores. b. Basidia. c. Cheilocystidia. d. Pleurocystidia. e. Hymenophoral trama. f. Pileipellis. Scale bars: a, b = 10 µm; c–e = 20 µm; f = 40 µm. Drawings by M. Gelardi.

TRAMA bilateral divergent ("*Phylloporus*-type"), hyphae very slightly divergent to nearly subparallel and tightly arranged, non-gelatinous [lateral strata hyphae in transversal section constricted at septa and distinctly broader than the mediostratum hyphae, touching or almost touching each other, (0–)2–4 µm apart, (7–)10–21(–24) µm diam., relatively thick-walled (≤1 µm)], hyaline in KOH, inamyloid in Melzer's; lateral strata (15–)20–40 µm thick, mediostratum 10–40 µm thick, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 4–12(–15) µm diam.; in Congo Red the mediostratum is concolorous with the lateral strata. RHIZOMORPHS consisting of parallel and densely arranged, unbranched, cylindrical, moderately thick–walled (≤1 µm), smooth hyphae, 5–10(–40) µm diam., yellowish-orange to reddish-brown in KOH, inamyloid to weakly dextrinoid in Melzer's. CLAMP CONNECTIONS absent in all tissues. HYPHAL SYSTEM monomitic. ONTOGENY gymnocarpic.

Ecology & distribution—Solitary or gregarious on soil in mixed forests dominated by *Fagaceae* (*Quercus*, *Castanopsis*, *Lithocarpus*) and *Pinaceae* (*Keteleeria, Pinus*); reported from southwestern China and Japan; distribution limits unknown.

Specimen examined: **CHINA**, **Yunnan Province**, **Xiaguan County**, Cangshan Mountain National Geopark, Gantong Temple, 25°39′03″N 100°09′58″E, 2260 m a.s.l., on a slope facing east in a subtropical montane forest hosting a country graveyard, a single mature specimen with *Pinus yunnanensis* Franch. and *P. armandii* Franch. with the presence of *Quercus variabilis* Blume, 19 Sep 2012, E. Horak & M. Gelardi (E. Horak 13448 (herb. ZT), MG484; GenBank KX911873, KX911874).

Associated fungi: Other fungal species found in the same habitat are *Amanita parvipantherina* Zhu L. Yang et al. and *Cantharellus appalachiensis* R.H. Petersen.

Discussion

The combination of morphological and molecular analyses confirmed our identification of *Hourangia cheoi*. This eye-catching species appears to be well outlined, especially due to the recent re-description provided by Zhu et al. (2015). Although somewhat variable in its appearance, *H. cheoi* is easily recognized in the field based on the following set of macroscopic characters: its 1) small to medium stature, 2) finely squamulose, ochraceous to brownish or reddish–brown pileus surface that slowly darkens when handled, 3) hymenophore that quickly blues when injured, 4) ≤2 mm diam. angular pores, 5) brownish to pale ochraceous fibrillose stipe that grades into beige towards the base, and 5) whitish context that in the pileus turns light blue, then pinkish-red, and finally grayish or dirty brown but in the stipe discolors directly reddish then brownish or grayish-black upon exposure. Diagnostic anatomical features include the 1) medium-sized boletoid basidiospores with a bacillate surface under SEM, 2) very large (≤113 × 23 µm) prominent pleurocystidia, and 3) trichodermal pileipellis comprising swollen cylindrical to sausage-shaped terminal cells up to 33 µm diam. (Chiu 1948, Zhu et al. 2015; pers. obs.).

Several accounts have treated extensively or cited *Hourangia cheoi* under the specific epithets "cheoi" or "punctilifer" (Zang 1986, 1996, 2006; Li & Song 2000, 2003; Wang 2004, Zhang et al. 2012, Zang et al. 2013), and it has generally been regarded as edible (Li & Song 2002, Dai et al. 2010), albeit in most cases the real identity of the mushroom in cited reports cannot be established with certainty.

The type species of *Xerocomus*, *X. subtomentosus* (L.) Quél., differs from *Hourangia cheoi* by its finely tomentose pileus, pale yellowish context with

pinkish hues in the lower third of the stipe and which turns light blue in the pileus and pileus/stipe connection zone but without an eventual reddish to brownish discoloration, stipe usually coarsely ribbed or roughly pseudoreticulate, trichodermal pileipellis consisting of narrow filamentous hyphae (terminal elements averaging 40 × 12 µm), obviously smaller pleurocystidia, temperate distribution, and obligate ectomycorrhizal association with broadleaved trees (mainly *Fagaceae* and *Corylaceae*) (Alessio 1985, Breitenbach & Kränzlin 1991, Engel et al. 1996, Lannoy & Estadès 2001, Ladurner & Simonini 2003, Watling & Hills 2005, Taylor et al. 2006, Klofac 2007, Šutara et al. 2009, Knudsen & Taylor 2012, Galli 2013).

For morphological comparisons of *H. cheoi* with the other three *Hourangia* species, consult the dichotomous key in Zhu et al. (2015).

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Bharatheeya coronata sp. nov., a conidial fungus from Brazil

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Abstract—*Bharatheeya coronata*, collected from decaying twigs in Bahia state, Brazil, is described and illustrated. The new species differs from both other *Bharatheeya* species by its smooth conidiophores, terminal conidiogenous cells that are truncate at the apex, and coronate, 1-distoseptate, pale brown conidia.

Key words—asexual ascomycete, hyphomycetes, taxonomy, tropical fungi

Introduction

The diversity of saprobic conidial fungi in tropical regions is still largely unknown, although the number of fungal inventories has increased in recent years (Maia et al. 2015). Several studies in different habitats in Brazil, especially in the semiarid region, have revealed novel taxa and new records of conidial fungi (Conceição et al. 2016, Fiuza et al. 2016, Monteiro et al. 2016). During a mycological survey of conidial fungi occurring on decaying plant material in the Brazilian semiarid region, an interesting specimen was found, clearly congeneric with *Bharatheeya* D'Souza & Bhat but different from the two previously described species. It is therefore described here as a new species.

Materials & methods

Samples of decaying plant material from Serra da Fumaça, Bahia, Brazil, were placed in paper bags. The materials were washed in tap water and placed on moist filter paper in glass Petri dishes (100 mm diam.). The dishes were then placed in plastic containers (150 L capacity), containing 200 mL sterile water and 2 mL glycerol, and incubated at room temperature (Castañeda-Ruiz et al. 2016). The plant material was regularly examined over a month of incubation for the presence of microfungi. Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were taken at ×1000. Microphotographs were made using a DP25 Olympus digital camera attached to a BX51 Olympus microscope equipped with bright field and Nomarski interference optics. The specimen was deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Bharatheeya coronata L.B. Conc., M.F.O. Marques, J.S. Monteiro, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 1

MycoBank MB819175

Differs from both other *Bharatheeya* spp. by its smooth conidiophores, its terminal conidiogenous cells that are truncate at the apex, and its coronate, 1-distoseptate, pale brown conidia.

Type: **Brazil**. Bahia State: Pindobaçu, Serra da Fumaça, 10°39′S 40°22′W, on decaying twig of unidentified plant, 8.Aug.2015, coll. L.B. Conceição (**Holotype**: HUEFS 216708).

Etymology: Latin, *coronata*, referring to the crown-like projections on the conidial apex.

Colonies on the natural substrate effuse, hairy, brown to pale brown. Mycelium superficial and immersed, composed of branched, cylindrical, septate, smooth, 2–3 µm diam., pale brown hyphae. Conidiophores macronematous, mononematous, single, erect, straight or slightly flexuous, unbranched, cylindrical, 2–5-septate, thick-walled, smooth, brown, paler toward the apex, 62.5–147.5 µm long, basal cell slightly inflated or sometimes radially lobed, 4–15 µm diam. Conidiogenous cells polytretic, integrated, terminal or rarely subterminal, cylindrical, truncate at the apex, smooth, pale brown, (9–)18–28 × 3–4 µm; 30–60 µm long, with or without percurrent extensions. Conidial secession schizolytic. Conidia solitary, acropleurogenous, 1-distoseptate, cuneiform, turbinate to broadly pyriform, dry, smooth, pale brown, 10–17 × 7–11 µm, with a small dark scar at the base, 1–2 µm diam., coronate at the apex with up to 10 projections.

Additional specimen examined: **BRAZIL. Bahia State:** Santa Teresinha, Serra da Jibóia, 10°74′S 40°36′W, on decaying wood of unidentified plant, 25.III.2009, F.R. Barbosa (HUEFS 216659).

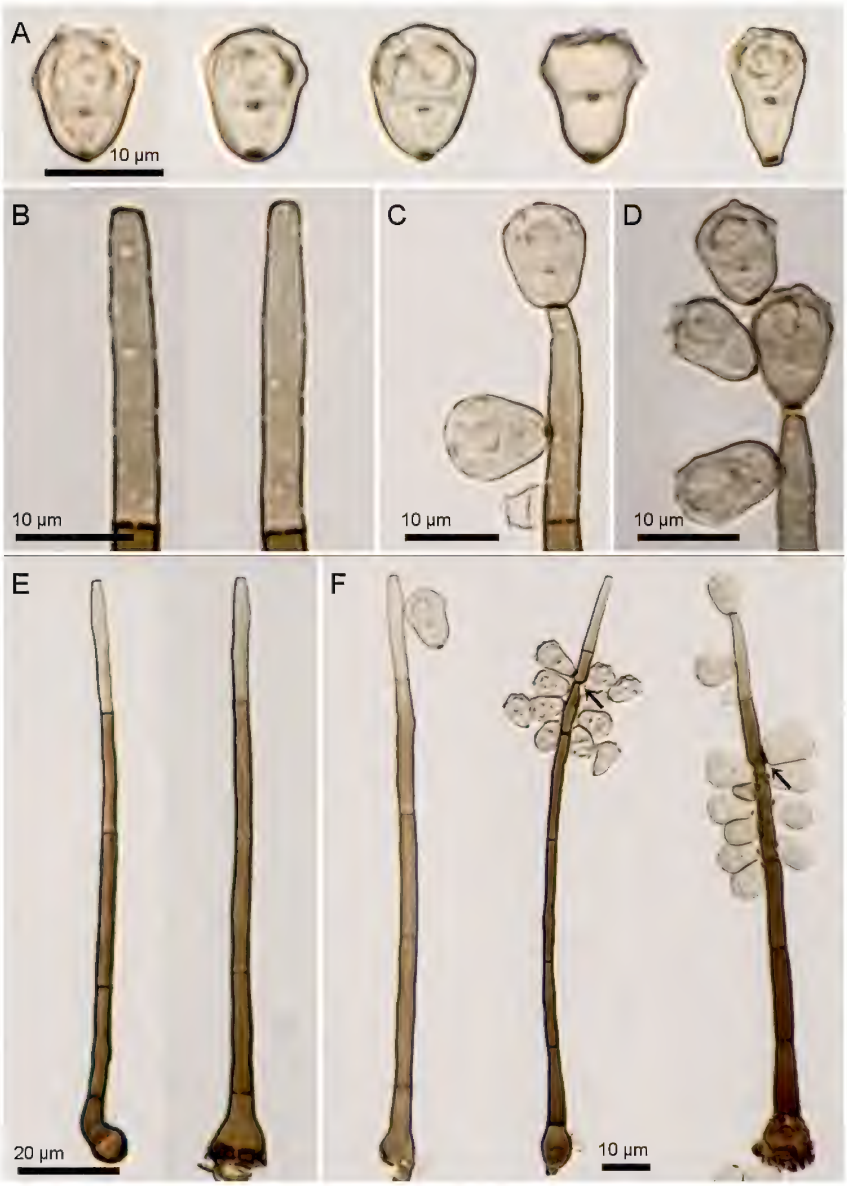

Fig 1. *Bharatheeya coronata*: (ex HUEFS 216708). A. Conidia. B. Conidiogenous cells. C, D. Conidiogenous cells and conidia. E. Conidiophores and conidiogenous cells. F. Conidiophores, conidiogenous cells, and conidia;. Arrows indicate the conidiogenous cell extensions.

NOTE: *Bharatheeya* was established by D'Souza & Bhat (2002) and typified by *Spadicoides goanensis* Bhat & W.B. Kendr. [≡ *B. goanensis* (Bhat & W.B. Kendr.) D'Souza & Bhat]. The genus is distinguished by conidiophores that are mononematous, smooth or verrucose, intercalary, and integrated, polytretic conidiogenous cells, and solitary, distoseptate, smooth to rough-walled conidia. *Bharatheeya coronata* shares polytretic conidial ontogeny and distoseptate conidia with *B. goanensis* and *B. mucoidea* D'Souza & Bhat. However, *B. goanensis* is distinguished by its verrucose conidiophores and 3-distoseptate conidia that are cylindrical-ovoid to obclavate (described inaccurately as "clavate" by D'Souza & Bhat 2002), while *B. mucoidea* is characterized by 2–4-distoseptate conidia (with globose, mucilaginous apical tunica) that are obpyriform to obclavate (inaccurately as "pyriform to clavate" in D'Souza & Bhat 2002).

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New records of *Phallales* from Paraguay

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Abstract—Four phalloid fungal species collected in the Central Department are reported for the first time from Paraguay: *Clathrus columnatus*, *Itajahya galericulata*, *I. rosea,* and *Mutinus argentinus*. Macro- and microscopic features are described for each species and photographs of fresh basidiomata are also provided. Additional comments address species distribution and taxonomy.

Key words—*Clathraceae*, gasteroid fungi, neotropical fungal diversity, *Phallaceae*, stinkhorns

Introduction

Gasteromycetes Fr. is no longer recognized as a formal class (Hibbett et al. 1997), and some of its members have been accommodated into the subclass *Phallomycetidae* K. Hosaka et al. within the orders *Gomphales* Jülich, *Phallales* E. Fisch., *Hysterangiales* K. Hosaka & Castellano, and *Geastrales* K. Hosaka & Castellano (Hosaka et al. 2006, Hibbett et al. 2007).

Within the *Phallales* spores are dispersed by insects, especially *Diptera* (Hibbett et al., 1997); *Phallaceae* Corda is a well defined family that embraces the commonly known "stinkhorns," which are characterized primarily by a simple hollow pseudostipe and a slimy spore mass (glebal mass) usually supported by a campanulate receptacle or spread over the pseudostipe surface (Burt 1896, Lloyd 1909, Cunningham 1944, Zeller 1949, Calonge 1998).

Several genera have been included in *Phallaceae*. Cunningham (1944) accepted seven genera: *Aporophallus* Möller, *Dictyophora* Desv., *Floccomutinus* Henn., *Itajahya* Möller, *Mutinus* Fr., *Phallus* L., and *Staheliomyces* E. Fisch. Zeller (1949) included another three: *Echinophallus* Henn., *Jansia* Penz., and *Xylophallus* (Schltdl.) E. Fisch. More recently, Kreisel (1996) treated *Aporophallus*, *Dictyophora*, and *Itajahya* as synonymous with *Phallus*, a taxonomic treatment that has been accepted for many years (Calonge 2005).

Although phylogenetic studies based on molecular data currently support *Dictyophora* within *Phallus* (Hosaka et al. 2006, Trierveiler-Pereira et al. 2014), however, they also support an independent generic status for *Itajahya* with two species: *I. galericulata* and *I. rosea*. *Itajahya* can be separated morphologically from other phalloid genera by the presence of a discoid structure (calyptra) that surrounds the receptacle apex and the morphology of the receptacle (Cabral et al. 2012, Marincowitz et al. 2015). The absence of receptacle and veil separate *Mutinus* species where the mucilaginous glebal mass spreads directly on the pseudostipe surface (Calonge 1998).

For many years, *Clathraceae* Chevall. has been regarded as separate from *Phallaceae* (e.g., Hawksworth et al. 1995: 581). Dring (1980) characterized taxa in the *Clathraceae* by basidiomes bearing the mucilaginous gleba on the inner surface of the receptacle, which may be lattice-shaped or consist of several columns. When the gleba is exposed to the exterior, then it is located on the surface of an apical lattice or column cluster. Although molecular studies support *Clathraceae* as a monophyletic group worthy of recognition at family level (Hosaka et al. 2006, Trierveiler-Pereira et al. 2014), *Phallaceae* sensu stricto and *Clathraceae* are still frequently treated as a single family, *Phallaceae* sensu lato (Kirk et al. 2001, 2008).

Mycological research in Paraguay was initiated by Spegazzini (1884, 1888, 1891), who reported 12 species from the country. More recent studies have been carried out by Campi and collaborators (Campi et al. 2012, 2015; Campi & Maubet 2015a,b). Here we report four new records of phalloid species from Paraguay: *Clathrus columnatus* (*Clathraceae*) and *Itajahya galericulata*, *I. rosea*, and *Mutinus argentinus* (*Phallaceae*).

Materials & methods

Specimens were collected in the Central Department, Paraguay, on the Universidad Nacional de Asunción campus and in the Asunción urban area. All specimens are deposited at the herbarium of the Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Asunción, Paraguay (FACEN).

Description of macroscopic features, as basidiomata size, shape and colors, were based on fresh specimens. For the microscopic analysis portions of the basidiomata were mounted in 5% KOH and examined with a Carl Zeiss compound microscope. Anatomical structures were dyed with phloxine and Congo red.

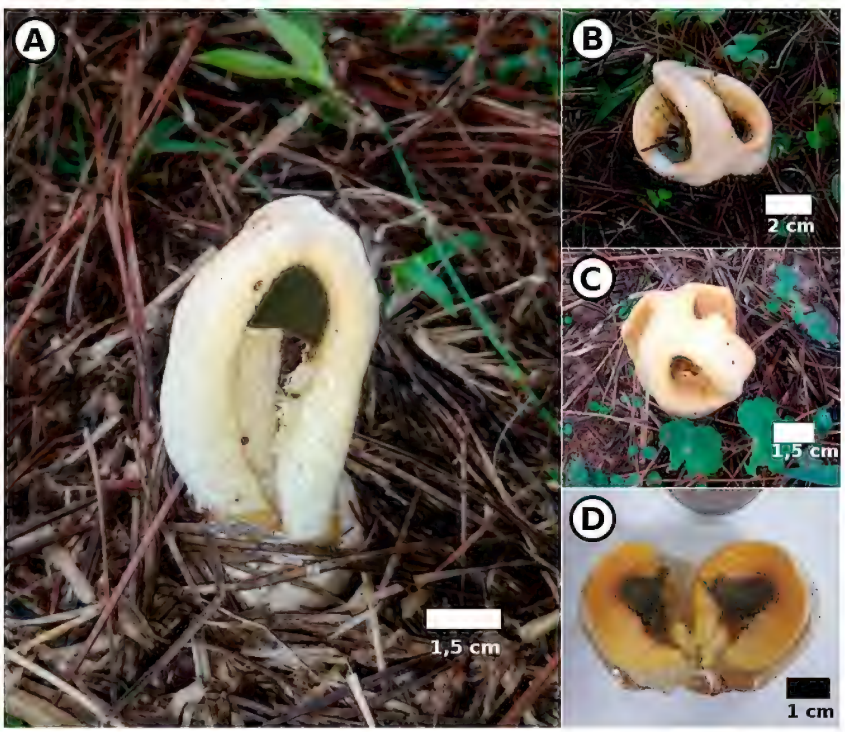

Fig 1. *Clathrus columnatus*.
A-C. Basidioma in situ growing near *Pinus*. D. Immature basidioma in transverse section.

Taxonomy

Clathrus columnatus Bosc, Mag. Gesell. Naturf. Freunde, Berlin 5: 85, 1811. Fig. 1

Immature basidiomata epigeous, globose to ovoid, 3.2–3.7 × 2.5–2.7 cm, attached to the substrate by rhizomorphs ≤7 cm long. Peridium of two layers, the outer membranous, <0.1 cm thick, whitish, smooth, covering the inner gelatinous layer, colorless, thickening (≤4 cm thick) towards the apex until only the exoperidium remains covering the columns of the mature basidioma. Dehiscing by irregular tears from the apex or occasionally

circumferentially. Expanded basidiome 6.8–10.3 cm high, whitish at the base, becoming yellowish to orange or reddish towards the apex, divided into 3–4 columns of 1.4–1.9 cm diam., spongy, free at the base, attached together at the apical zone by a transverse column. Gleba confined in the inner portion of the apex, olivaceous and mucilaginous, odor fetid.

Basidiospores 4–5 × 1.3–2 µm, cylindrical, hyaline to greenish in KOH, congophilic, with 1–2 internal guttules at the ends, thin-walled, smooth. Columns composed of pseudoparenchymatous, irregularly rounded cells, 16–35 µm diam. Exoperidium composed of septate hyphae, 4.5–7.5 µm diam., thin-walled, branched; the inner gelatinous layer composed of thin-walled hyphae, (2–)3–4 µm diam., hyaline; septa with clamp connections.

Ecology & distribution—solitary or in small groups, appearing soon after rain in cold temperatures on soil among litter in a *Pinus elliottii* Engelm. plantation in an urban setting.

Specimens examined—**PARAGUAY. Central Department, San Lorenzo,** 25°20′13″S 57°31′33″W, 26.V.2016, leg. Y. Maubet N° 16 (FACEN 3343).

Distribution in South America—Brazil (Trierveiler-Pereira & Baseia 2009, Magnago et al. 2013) and Chile (Sandoval-Leiva et al. 2014). This is the first record of *C. columnatus* from Paraguay.

Comments—Dring (1980) noted that a variety of forms can be found with respect to the number of arms and the general shape of the receptacle. In North American specimens, a transverse arm connects the vertical arms, as in our material. Dring (1980) also observed that the type description of *Clathrus australis* Speg. agrees with the type description of *C. columnatus* except for the presence of a fifth column. Unfortunately, as the Argentinean type material *C. australis* could not be found, some taxonomic doubt remains regarding the separation of the two species. Spegazzini (1887a) stated that he also had samples of *C. australis* from Paraguay, but they were in very bad condition. Spegazzini (1887b) later retained both *C. columnatus* and *C. australis* from Brazil as independent species.

Sandoval-Leiva et al. (2014) and Magnago et al. (2013) characterize the species as having 2–5 robust spongy reddish to orange columns free at the base and fused at the apex and a gleba that is spread on the internal portion of the columns and not confined to a glebifer. Based on the description and illustrations presented by previous authors (Dring 1980, Trierveiler-Pereira 2015) we conclude that our material represents *C. columnatus*.

Fig. 2. *Itajahya galericulata*. A. Basidioma in situ growing near *Guadua*. B. Expanded basidioma and immature stage in transverse section.

Itajahya galericulata Möller, Bras. Pilzbl.: 79, 148, 1895. Fig. 2

Immature basidiomata epigeous, subglobose to globose, 4–5 × 3.5–4 cm, exoperidium ochre to dun, slightly leathery at the apex. Basal mycelial cord thick, 3–5 cm long and 0.2–0.4 cm thick. In transverse section, the peridium has four distinguishable layers with the continuous mucilaginous layer located between the other layers between the peridium and gleba. Expanded basidioma phalloid in shape. Stipe white, hollow, spongy and

externally longitudinally striated, 13.5–20 cm high × 2–4 cm diam., tapering towards the base. Receptacle cylindrical with grooved surface, up to 3.5 × 2.5 cm, covered with the mucilaginous gleba. Gleba deliquescent, dark green, odor putrid; calyptra at the receptacle apex white, discoid, flattened, 2–3 cm in diam., spongy, forming a sterile platform above the receptacle.

Basidiospores 5–6 × 2–3 µm, cylindrical, hyaline to slightly light green in KOH, thin-walled, smooth. Receptacle hyphae pseudoparenchymatous, globose to cylindrical, 400–100 × 20–45 µm; calyptra hyphae pseudoparenchymatous (globose to cylindrical, 33–70 × 24–34 µm) and thin-walled (septate, clamped, 2–13 µm diam.). Mycelial cord hyphae thin-walled, septate, clamped, 3–5 µm diam.

Ecology & distribution—Gregarious, fruiting soon after heavy rain during summer on soil among fallen litter and undergrowth in an urban area.

Specimens examined—**PARAGUAY. Central Department, Asunción**, 25°18′23″S 57°35′41″W, 21.I.2014, leg. Y. Maubet N° 38 (FACEN 3424).

Distribution in South America—Brazil (Möller 1895, Rick 1961), Argentina (Spegazzini 1927, Fries 1909, Wright 1949, Ruíz Leal 1954, Domínguez de Toledo 1995, Hernández Caffot et al. 2015), and Bolivia (Rocabado et al. 2007). This is the first record of *I. galericulata* from Paraguay.

Comments—Möller (1895) stated that in South America *Itajahya* basidiomata are very variable in size and development at the edge of the receptacle and calyptra, unlike specimens found in North America, where they appear more stable (Long & Stouffer 1943). Domínguez de Toledo (1995) observed that in its "egg phase" the *I. galericulata* peridium consists of four layers around the gleba: two firm layers surrounding a mucilaginous and continuous layer, the same arrangement as we found in our specimen. We also observed longitudinal stripes in the stipe, a remarkable character that has not been noted in the literature. The morphological feature separating *I. galericulata* from *I. rosea* is the color of the pseudostipe, which is pinkish in the latter.

Marincowitz et al. (2015) reported an association between *I. galericulata* and the tree species *Jacaranda mimosifolia* D. Don in Pretoria, South Africa. In Brazil, Bolivia, and Paraguay, specimens were collected in pastures of urban areas in accordance with the ecology noted by Domínguez Toledo (1995) and Hernández Caffot et al. (2015), who described *I. galericulata* growing in compacted soils with low grasses during summer and autumn.

Fig. 3. *Itajahya rosea*. A. Basidioma in situ growing among grass. B. Immature stage in transverse section and expanded basidioma.

Itajahya rosea (Delile) E. Fisch., Ber. Deutsch. Bot. Ges. 47: 294, 1929 Fig. 3

Immature basidiomata epigeous, subglobose to globose, 1.4–1.7 × 3–3.4 cm, exoperidium ochre to dun, slightly leathery at the apex. In transverse section, it is possible to observe that the pseudostipe is pink and surrounded by three layers: outer layer 0.3 cm thick, firm, white to beige, crackled; middle layer 0.8 cm thick, mucilaginous, aqueous, hyaline; inner layer >0.1 cm thick, whitish, dry, covering the green gleba. Expanded basidioma Stipe white, hollow, fibrous, 9.5–14.2 × 1.6–1.8 cm, slightly tapering towards the base. Receptaculum conic with reticulated surface, 2 × 1 cm, covered with gleba. Gleba mucilaginous, deliquescent, dark green, odor fetid; calyptra at the apex of the receptaculum white, discoid, flattened, 1.1–1.9 cm in diam., forming a sterile platform above the receptacle.

Basidiospores 4 × 2 µm, cylindrical, hyaline, slightly green, thin-walled, smooth. Stipe hyphae pseudoparenchymatous, globose, 15–25(–35) ×

22–35(–46) µm. Receptacle hyphae pseudoparenchymatous, globose to cylindrical, 12–23(–26) × 8–14(–16) µm; calyptra hyphae thin-walled, septate, clamped, 3–5(–6) µm diam.

Ecology & distribution—Gregarious, appearing soon after heavy rain during summer.

Specimens examined—**PARAGUAY. Central Department, San Lorenzo,** Campus Universitario, 26°20′08″S 57°31′14″W, I.2016, leg. Y. Maubet N° 002 (FACEN 3338).

Distribution in South America—Northeastern Brazil (Ottoni et al. 2010, Cabral et al. 2012). This is the first record of *I. rosea* from Paraguay.

Comments—According to Ottoni et al. (2010), the most relevant characteristics for the taxonomical determination of *I. rosea* is the presence of a calyptra at the apex of the receptacle and the pinkish pseudostipe. The species is morphologically very close to *I. galericulata* (see comments above). The peridium of the immature basidiome is another important feature separating the species: three-layered in *I. rosea* and four-layered in *I. galericulata*.

Moreno et al. (2013) recognized *I. rosea* as a rare species characteristic of desert and semiarid regions, as reported from Yemen (Kreisel & Al-Fatimi 2008) and Northeastern Brazil (Ottoni et al. 2010). However, our specimen was found in eastern Paraguay characterized by a subtropical climate with the rainy season occurring in the summer.

Future molecular research is needed to separate the two morphologically similar species.

Mutinus argentinus Speg., Anal. Soc. Cient. Argent. 24: 62, 1887. Fig. 4

Immature basidiomata epigeous, ellipsoid to ovoid, 3.0 × 1.8 cm, whitish, with basal rhizomorphs 3–6 cm long, fibrous, strongly attached to the substratum. In transverse section releasing an odorless translucent liquid; three layers surrounding the pseudostipe: the outer layer 0.1 cm thick, whitish to cream, covered with brownish fibers; medium layer 0.4 cm thick, wet, sticky, hyaline; inner layer >0.1 cm, very thin, whitish, enclosing the gleba. Expanded basidiome Stipe 6.6 × 0.9 cm, spongy, whitish at the base, becoming pinkish towards the apex, and finally bright red to maroon at the apex, apex with a small pore. Gleba mucilaginous, olive green, covering the stipe apex.

Basidiospores 5–7 × 2–3 µm, cylindrical, hyaline, slightly green, thin-walled, smooth. Stipe hyphae pseudoparenchymatous, globose, 35–50 × 50–70 µm, Congo-Red negative. Glebal region hyphae pseudoparenchymatous, cylindrical, spherical to irregular in shape, 30–50 × 25–30 µm, pinkish.

Fig. 4. *Mutinus argentinus.*
A. Basidiome in situ on dead branch, among leaves. B. Immature stage on decaying wood and expanded basidiome and immature stage in transverse section.

Ecology & distribution—Growing solitary or in small groups of a few basidiomata on sandy soil among decaying fallen litter and wood debris.

Specimens examined—**PARAGUAY. Central Department**, San Lorenzo, Campus Universitário, 26°20′08″S 57°31′14″W, 23.III.2014, leg. M. Campi N° 45 (FACEN 3356).

Distribution in South America—Argentina (Spegazzini 1887a, 1906; Wright 1949) and Brazil (Meijer 2006, Magnago et al. 2013). This is the first record of *M. argentinus* from Paraguay.

Comments—Magnago et al. (2013) discussed the similarity of *M. argentinus* and *M. bambusinus* (Zoll.) E. Fisch., two species that have been treated as synonyms by several authors. Dring & Rose (1977) presented the morphological differences between the two species: *M. bambusinus* produces a larger fertile portion that is strongly pigmented and with a sterile tip, while *M. argentinus* lacks the marked sterile tip. The species is also depicted by Trierveiler-Pereira (2015). Based on this information and the provisional *Mutinus* key by Silva et al. (2015), we conclude that our specimen represents *M. argentinus*.

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Arthrotaeniolella aquatica gen. & sp. nov. and *Pseudospiropes piatanensis* sp. nov. from Brazil

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Abstract—A new genus and species, *Arthrotaeniolella aquatica*, and a new species, *Pseudospiropes piatanensis*, collected on submerged decaying plant materials in the Brazilian semiarid region, are described and illustrated. *Arthrotaeniolella aquatica* is characterized by distinct, sinuate, slender, branched, hyaline or subhyaline to pale dirty brown conidiophores that produce cylindrical to sub-oblong, unicellular to euseptate, dark brown conidia formed by thallic-arthric disarticulation of the branches. *Pseudospiropes piatanensis* is distinguished by a polyblastic conidiogenous cell that produces fusiform to lunate, 10–15-distoseptate, pale brown conidia.

Key words—*Ascomycota*, hyphomycetes, taxonomy, tropics

Introduction

The Brazilian semiarid region, located in the northeast of the country, covers approximately 900,000 km^2 and has high biodiversity (Giulietti et al. 2006). Its different vegetation types have favored a great diversity of microfungi with the presence of several new species (e.g., Barbosa et al. 2013, Silva & Gusmão 2013, Almeida et al. 2014, Silva et al. 2014, Monteiro et al. 2016). During continuing mycological surveys of microfungi from this region, two interesting hyphomycetes were collected on decaying plant materials submerged in a stream. Close examination of their morphological

characteristics revealed that one clearly belongs to the genus *Pseudospiropes* M.B. Ellis (Ellis 1971). The other, which shows remarkable differences from all previously described hyphomycetes (Seifert et al. 2011), required the erection of a new genus, *Arthrotaeniolella*.

Materials & methods

Samples of submerged litter were placed in plastic bags, taken to the laboratory, placed in Petri dish moist chambers, and stored in a 170 L polystyrene box with 200 mL water plus 2 mL glycerol at room temperature for 30 days (Castañeda-Ruiz et al. 2016). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements were made at a magnification of ×1000. Microphotographs were obtained with an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. The type specimens are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Arthrotaeniolella L.B. Conc., M.F.O. Marques, J.S. Monteiro, Gusmão & R.F. Castañeda, **gen. nov.**

MycoBank MB 821602

Differs from *Taeniolella* by its production of conidia by thallic-arthric disarticulation of conidiophore branches.

Type Species: *Arthrotaeniolella aquatica* L.B. Conc. et al.

Etymology: Greek, *arthro-*, meaning jointed, referring to the arthric-thallic conidia formed by breaking up fertile branches at the septa; *-taeniolella*, referring to the genus *Taeniolella*.

Conidiophores macronematous, mononematous, branched, septate, subhyaline or pigmented. Conidiogenous hyphae holothallic, pigmented. Conidial secession schizolytic. Conidia solitary, cylindrical to sub-oblong, unicellular or euseptate, dark pigmented, formed by disarticulation of the conidiogenous branches.

Arthrotaeniolella aquatica L.B. Conc., M.F.O. Marques, J.S. Monteiro, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 1

MycoBank MB 821603

Differs from *Taeniolella rudis* by its alternately arranged branches conidiophores and its random thallic-arthric conidial ontogeny

Type: Brazil, Bahia State, Pindobaçu, Serra da Fumaça, 40°21′W 10°39′S, on submerged decaying leaves and twigs of unidentified plant in a stream, 26.July 2016, coll. L.B. Conceição (**Holotype**, HUEFS 224988).

Etymology: Latin, *aquatica,* referring to its growing in water.

Fig. 1. *Arthrotaeniolella aquatica* (holotype, HUEFS 224988). A. On natural substrate; B. General aspect; C, D. Conidia; E, F. Disarticulating conidia. G. Disarticulated conidia. Scale bars: A = 100 µm; B–G = 10 µm.

Colonies on natural substrate effuse, hairy, brown. Mycelium mostly superficial, hyphae branched, septate, 1–2 µm diam., smooth, subhyaline to pale dirty brown. Conidiophores macronematous, mononematous, slender, erect, flexuous or straight, subhyaline or pale dirty brown, 55–250 × 6–9 µm; sometimes unbranched but mostly with dark dirty brown, 4–7-septate, 40–80 µm long branches, alternately arranged toward the apex Conidiogenous hyphae holothallic, branches and differentiated apical region of the unbranched conidiophores cylindrical to long clavate, mostly discrete, sometimes integrated, dark dirty brown. Conidia cylindrical, sub-oblong, truncated at the ends, occasionally rounded at the apex, 0–4-septate, 8–35(–40) × 3–9 µm, dark dirty brown, dry, smooth, forming by random thallic-arthric disarticulation at the septa of the conidiogenous hyphae.

Note: *Taeniolella* S. Hughes is characterized by conidiophores that are little differentiated from hyphae, usually short, unbranched or sparingly branched near the base and conidiogenous cells that are monoblastic, integrated, terminal, cylindrical or doliiform and that produce conidia that are solitary or in branched acropetal chains, 1 or more septate, often seceding with difficulty, cylindrical, dark brown (Hughes 1958, Ellis 1971, Seifert et al. 2011). In *T. rudis* (Sacc.) S. Hughes the conidial chains are broken up by disarticulation (Jones et al. 2002; Seifert et al. 2011). In *Arthrotaeniolella* the conidiogenous events occurring in the branches and hyphae are classified as conidial development type 38 (conidial ontogeny holothallic, conidiogenous cells formed by apical wall-building coincident with conidial ontogeny, random delimitation by 1 septum at each end, no maturation during conidiogenesis, secession randomly schizolytic) in Kirk et al. (2008), which is different from the blastic conidial ontogeny in *Taeniolella*.

Two other genera illustrated by Seifert et al. (2011)—*Ampulliferina* B. Sutton and *Thielaviopsis* Went—have similar thallic-arthric conidial development in the branches. In *Ampulliferina* the superficial hyphae are hyphopodiate, with lateral, globose, clavate, rarely lobate, brown hyphopodia (Sutton 1969, Seifert et al. 2011). In *Thielaviopsis* the thallic-arthric development is restricted to the chlamydospores-like branches, which are distinguished by the production of unicellular or septate, dark brown arthroconidia with a germ slit or pore (Seifert et al. 2011).

Fig. 2. *Pseudospiropes piatanensis* (holotype, HUEFS 211342). A. Conidia; B–D. Conidiophores; E. Detail of conidiogenous cell; F. Conidiogenous cell and conidia. Scale bars = 10 µm.

Pseudospiropes piatanensis J.S. Monteiro, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 2

MycoBank MB 821604

Differs from *Pseudospiropes mussaendae* and *P. ximeniae* by its 10–15-distoseptate fusiform to lunate conidia.

Type: Brazil, Bahia State: Piatã, Rio Coxó, 13°00′S 41°53′W, on submerged decaying wood in a stream, 9 May 2014, coll. J.S. Monteiro (**Holotype**, HUEFS 211342).

Etymology: *piatanensis*, referring to the City of Piatã where the type was found.

Colonies on natural substrate effuse, hairy, brown. Mycelium mostly immersed, hyphae branched, septate, smooth, pale brown, 1–2 µm diam. Conidiophores macronematous, mononematous, single, simple, erect, straight or slightly flexuous, septate, smooth, dark brown, pale brown toward the apex, 82.5–87.5 × 4–5 µm. Conidiogenous cells holoblastic, polyblastic, indeterminate, integrated, terminal (becoming intercalary), sympodial, cylindrical, smooth, pale brown, 27.5–52.5 × 6–7.5 µm. Conidiogenous loci enlarged, thickened, protuberant, melanized, lenticular. Conidial secession schizolytic. Conidia solitary, acropleurogenous, fusiform to lunate, 10–15-distoseptate, smooth, pale brown, 57.5–72.5 × 10–16 µm, 2.5–3 µm diam. at the truncate base.

Additional specimen examined: **BRAZIL, Bahia: Piatã, Rio Coxó,** 13°00′S 41°53′W, on submerged decaying wood in a stream, 9 May 2014, coll. J.S. Monteiro (HUEFS 211343).

Note: *Pseudospiropes* is distinguished by solitary, acropleurogenous, distoseptate conidia seceding schizolytically from polyblastic, sympodial, cicatrized conidiogenous cells with enlarged, thickened, protuberant and melanized conidiogenous loci (Ellis 1971, Castañeda-Ruiz et al. 2001, Seifert et al. 2011). The 17 species currently included in this genus are delimited by conidial shape, size, number of septa, and pigmentation (Castañeda et al. 2001, Ma et al. 2011). Among *Pseudospiropes* species, only *P. mussaendae* Z.Q. Shang & X.G. Zhang and *P. ximeniae* Z.Q. Shang & X.G. Zhang have conidia similar to those of *P. piatanensis*. However, the conidia of *P. piatanensis* have more distosepta and are longer than those of *P. mussaendae* (conidia 36–47 × 9–11 µm, 8–9-distoseptate) and *P. ximeniae* (conidia 34–40 × 12–14 µm, 6–8-distoseptate). In addition, the conidia of *P. piatanensis* are fusiform to lunate, while those of *P. mussaendae* are fusiform to obclavate and those of *P. ximeniae* fusiform to navicular (Shang & Zhang 2007).

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Cunninghamella clavata from Brazil: a new record for the western hemisphere

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Abstract—During a survey of *Mucorales* in Brejo dos Cavalos (a fragment of an Upland Atlantic Forest within the semi-arid region of Northeast Brazil) a specimen of *Cunninghamella clavata* was isolated from soil samples. A detailed description of the specimen and a phylogenetic analysis of its relationship with other *Cunninghamella* species are presented. An identification key for *Cunninghamella* taxa reported from Brazil is also provided.

Key words—*Mucoromycota*, *Cunninghamellaceae*, LSU rDNA

Introduction

Cunninghamella Matr. (*Cunninghamellaceae* Naumov ex R.K. Benj., *Mucorales* Fr.) comprises species characterized by the production of sporophores with pedicellate unispored sporangiola covered with spines (Zheng & Chen 2001). The sporophores are irregularly, verticillately, or pseudoverticillately branched, and the *Mucor*-like zygospores present a reddish-brown wall (Baijal & Mehrotra 1980, Benny 2006). *Cunninghamella* species have a worldwide distribution, and most are saprobes, often found in soil, stored grains, and other organic substrates (Baijal & Mehrotra 1980, Yu et al. 2015). Some species are pathogenic to humans, particularly immunocompromised patients (Hsieh et al. 2013).

Species of *Cunninghamella* have traditionally been distinguished based on morphological features, such as colony color and texture, pattern of sporophore

branching, vesicle shape and size, sporangiola shape, size, and color, and the presence/absence and length of the projections (spines) in the sporangiola (Zheng & Chen 2001). *Cunninghamella* has been monographed by several authors (Alcorn & Yeager 1938, Cutter 1946, Milko & Beljakova 1967, Samson 1969, Baijal & Mehrotra 1980, Lunn & Shipton 1983) based on morphological characteristics. A subsequent monograph by Zheng & Chen (2001) based on morphological characteristics, maximum growth temperatures, mating experiments, and the length of the ITS region (RFLP analysis) recognized twelve species and three varieties: *C. bertholletiae* Stadel, *C. blakesleeana* Lendn., *C. binariae* R.Y. Zheng, *C. clavata*, *C. echinulata* var. *antarctica* (Caretta & Piont.) R.Y. Zheng & G.Q. Chen, *C. echinulata* (Thaxt.) Thaxt. ex Blakeslee var. *echinulata*, *C. echinulat*a var. *nodosa* R.Y. Zheng, *C. echinulata* var. *verticillata* (F.S. Paine) R.Y. Zheng & G.Q. Chen, *C. elegans* Lendn., *C. homothallica* Komin. & Tubaki, *C. intermedia* K.B. Deshp. & Mantri, *C. multiverticillata* R.Y. Zheng & G.Q. Chen, *C. phaeospora* Boedijn, *C. septata* R.Y. Zheng, and *C. vesiculosa* P.C. Misra. Liu et al. (2001) and Yu et al. (2015), who sequenced the ITS rDNA and tef-1α gene regions of taxa accepted by Zheng & Chen (2001), found a phylogeny that was consistent with Zheng & Chen's taxonomic conclusions. Subsequently, two additional species have been described: *C. bigelovii* Z. Xin et al. from China (Guo et al. 2015) and *C. gigacellularis* A.L. Santiago et al. from Brazil (Santiago et al. 2016).

Cunninghamella clavata has previously been reported only from Guizhou and Yunnan in southwest China, where the holotype and two paratype specimens were collected in 1985–92 (Zheng & Chen 1998). Here we present a detailed description of a *C. clavata* collection isolated from soil samples from an upland forest area in the semiarid region of Brazil. An identification key for *Cunninghamella* taxa recorded in Brazil and results of phylogenetic analysis of *Cunninghamella* LSU rDNA sequences are also provided.

Material & methods

Soil collection—Samples of soil were collected in September 2014 in Brazil's Brejo dos Cavalos Upland Atlantic Forest (8°16′S 35°58′W), located in the city of Caruaru in Pernambuco state, 130 km from Recife. The mean annual temperature is 22 °C, with rainfall ranging from 650 to 800 mm per year. The vegetation consists of montane ombrophilous forest, a remnant of the Atlantic Rainforest. The samples were placed in clean plastic bags and stored in Styrofoam boxes for transportation to the laboratory.

Isolation, purification, and identification of *C. clavata*—Portions of 5 mg of soil were added to Petri dishes containing a wheat germ agar culture medium

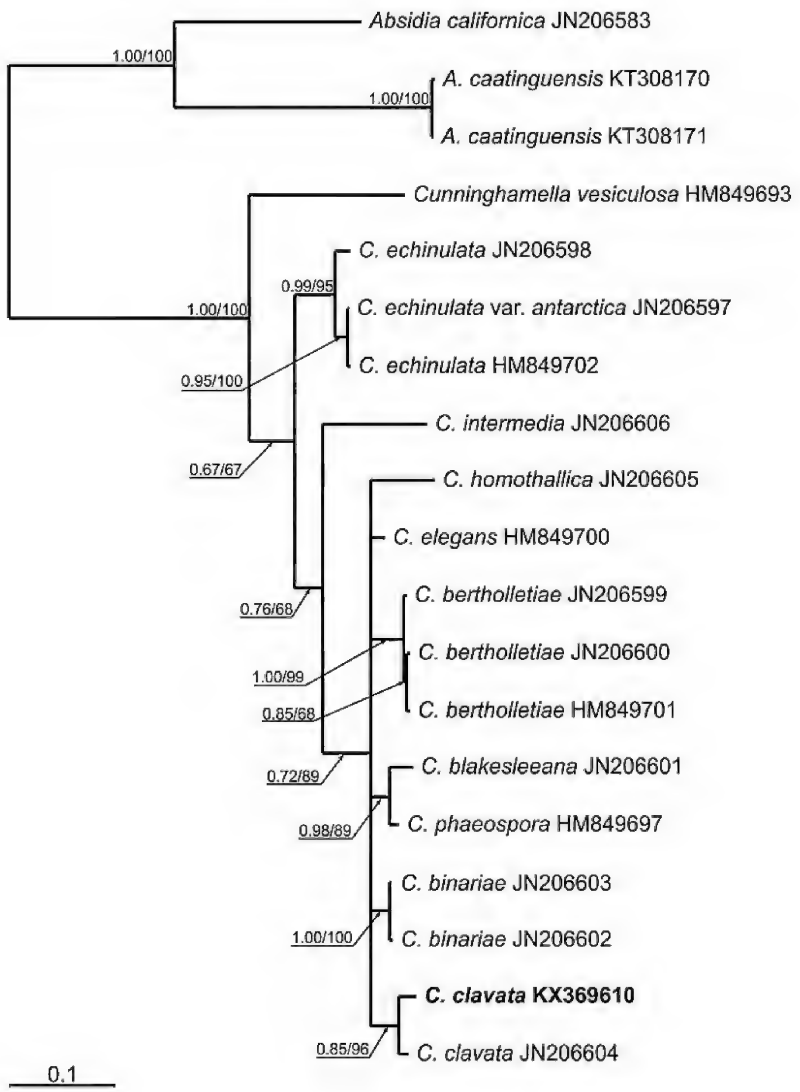

FIG. 1. Phylogenetic tree of *Cunninghamella* taxa, constructed using the large subunit (LSU) rDNA sequences, with *Absidia californica* and *A. caatinguensis* as outgroup. Sequences are labeled with their GenBank accession numbers. Support values are from Bayesian inference and maximum likelihood analyses. The sequence obtained in this study is in boldface.

(Benny 2008) augmented with chloramphenicol (NeoFenicol—Neo Química; 100 $mg.L^{-1}$). The dishes were stored on the laboratory bench at room temperature (26 ± 2 °C) for seven days and subjected to alternate light and dark periods. Fragments of mycelium were removed from the Petri dishes under a stereomicroscope (Leica EZ4) and transferred to Petri dishes with malt extract agar (MEA) (Benny 2008). The specimen was identified by comparing the macroscopic (colony color, appearance, and diameter) and microscopic (e.g., shape and size of sporophores, columellae, sporangia, and sporangiospores) characteristics with the descriptions of Zheng & Chen (1998, 2001). A voucher was conserved in the Micoteca, Federal University of Pernambuco, Recife, Brazil (URM).

Molecular analysis—cultures grown in test tubes containing MEA were incubated at 28 °C for six days to obtain fungal biomass. After the material was transferred to 2 mL microtubes with screw caps, 0.5 g acid-washed glass beads of two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA) were added to each tube. The material was crushed through stirring at high speed in a FastPrep homogenizer. The genomic DNA was extracted as described by Góes-Neto et al. (2005). The mycelium was washed with chloroform: isoamyl alcohol (24:1) and then homogenized in 2% cetyltrimethylammonium bromide buffer. The DNA was precipitated in isopropanol, washed with 70% ethanol, and resuspended in 50 µL of ultrapure water.

The primer pairs LR1/LSU2 were used for the amplification of the large subunit (LSU) of nuclear ribosomal DNA (rDNA) (Van Tuinen et al. 1998, Santiago et al. 2014). The polymerase chain reactions were carried out as described by Oliveira et al. (2014). The newly obtained sequences were deposited in GenBank database.

Phylogenetic reconstructions were obtained by analyzing the sequence data of the partial LSU rDNA gene. The fungal sequences were aligned with ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). Prior to phylogenetic analysis, the nucleotide substitution optimal model was estimated using TOPALi 2.5 (Milne et al. 2004). Bayesian inference (two runs over 1×10^6 generations with a burn-in of 2500) and maximum likelihood (with support estimated by a bootstrap analysis with 1000 replicates) analyses were performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from TOPALi 2.5.

Phylogenetic analysis

In BLASTn analysis the *C. clavata* sequence from our collection (URM 7410, KX369610) showed 98% identity with the sequence of the *C. clavata* type culture (CBS 100178, JN206604); these two sequences nested together in the phylogenetic tree (Fig. 1).

Taxonomy

Cunninghamella clavata R.Y. Zheng & G.Q. Chen, Mycotaxon 69: 189 (1998) Fig. 2

Colony initially white; after 4 days in MEA at 25 °C becoming pale

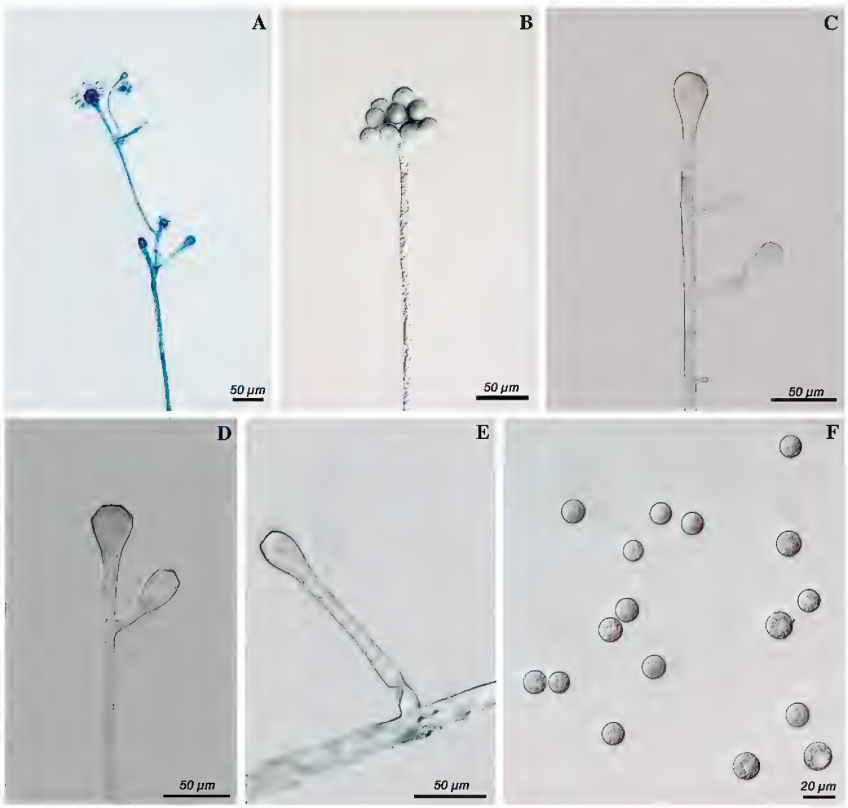

Fig. 2. *Cunninghamella clavata* (URM 7410): A. Repeatedly branched sporophore; B. Sporophore simple with sporangiola; C, D. Sporophores with lateral branches and vesicles; E. Unbranched sporophore with a vesicle; F. Sporangiola.

grayish with cream reverse and reaching 9.4 cm diam. and 9 mm high. Rhizoids abundant, long and short, simple or slightly branched. Stolons present, coenocytic, some showing septa near the sporophore origin. Sporophores short and long, erect or recumbent, arising from stolons or directly from aerial hyphae, hyaline, smooth-walled; some containing one or more septa below the sporangium or near the basis. Main sporophore axes simple or branched, usually equal in width throughout, some enlarging in the apical direction and ending in a vesicle (3.5–)5–10(–14) µm diam.; swelling below the vesicle present. Primary branches simple, in pairs,

pseudo-verticillate (≤4). Short and long branches in the same sporophore common, repeatedly (≤6×) branching, some arising close to the vesicles and growing parallel to the main sporophores ≤500 × 7.5 µm, erect or slightly curved and terminating in a vesicle. Vesicles of the main sporophores light gray, mostly clavate, commonly irregular, angular, or flattened at the apex, rarely globose or subglobose, 17–47.5 × 20–37.5 µm. Lateral vesicles with same shape as the main vesicles, (2.5–)7.5–35 × (2.5)5.5–20(–25) µm. Sporangiola globose, light yellow with greenish yellow content and echinulate, (6.5–)10–20(–23) µm diam. Sporangiospores hyaline, echinulate-walled, usually remaining within the sporangiola. Chlamydospores and Zygosporangia not observed.

Habitat: isolated from Upland Atlantic Forest soil from the semiarid region of Pernambuco, northeastern Brazil.

Specimen examined: **BRAZIL, Pernambuco,** Caruaru: Brejo dos Cavalos, (8°16′S 35°58′W), in soil, 29.01.2015, leg. A.L. Alves (URM 7410; GenBank KX369610).

Geographic distribution: Guizhou and Yunnan, China (Zheng & Chen 1998) and Pernambuco, Brazil. This is the first report of *C. clavata* in the western hemisphere.

Discussion

Álvarez et al. (2011) and Walther et al. (2013) noted that the LSU rDNA region is a reliable indication of taxonomic differentiation in *Mucorales*. Our phylogenetic and morphological analyses support *C. clavata* as distinct from the other described *Cunninghamella* species. Morphologically, *C. clavata* is distinguished by producing clavate vesicles and the formation of repeatedly branching short and long branches within the same sporophore—some arising close to the vesicles and growing parallel to the main sporophores (Zheng & Chen 1998).

Cunninghamella echinulata var. *echinulata* also presents clavate vesicles, but lacks the exclusively globose sporangiola observed in *C. clavata*, and in *C. echinulata* var. *echinulata* colonies are white to pale, not pale grayish as in *C. clavata* (Zheng & Chen 2001).

Cunninghamella intermedia also produces strictly globose sporangiola, but its sporophores are not repeatedly branched and the vesicles are globose to subglobose, never clavate. The phylogenetic analysis of the LSU rDNA region confirmed the identity of our specimen.

Our Brazilian specimen represents the first western hemisphere record of *C. clavata* previously reported only from southwest China (Zheng & Chen

1998), contributing to our knowledge of *Mucorales* distribution. In Brazil, other species of *Cunninghamella* such as *C. bertholletiae*, *C. blakesleeana*, *C. echinulata* var. *echinulata*, *C. echinulata* var. *verticillata*, *C. elegans*, and *C. phaeospora* have been isolated from soil and from herbivore dung (Trufem 1981; Santiago et al. 2011, 2013).

Key to *Cunninghamella* taxa registered in Brazil

1. Colonies white to pale or turning yellowish; giant dark sporangiola present or not 2
1. Colonies pale grayish to deep or brownish gray; giant dark sporangiola not present 4
2. Colonies floccose, pale yellowish; sporangiola ovoid or ellipsoid (≤30 × 15 µm) or globose (≤17 µm diam.); giant dark sporangiola not present; maximum growth temperature 38 °C ..... ***C. blakesleeana***
2. Colony floccose or granulate, white to pale; sporangiola <22 × 18 µm; giant dark sporangiola present; maximum growth temperature 42 °C 3
3. Colony floccose; sporophores usually branched (pseudo)verticillately with shorter branches on the same sporophore rarely re-branching ..... ***C. echinulata* var. *verticillata***
3. Colony granulate; sporophores never (pseudo)verticillately branched; with shorter branches on the same sporophore often re-branching ..... ***C. echinulata* var. *echinulata***
4. Main sporophore axis terminating in a vesicle; long and short branches on the same sporophore present or absent, if present re-branching ≤6× 5
4. Sporophores terminating in a vesicle or dividing into apical branches; long and short branches in the same sporophore common, re-branching ≤3× ..... ***C. bertholletiae***
5. Vesicles clavate; short and long branches in the same sporophore repeatedly branching ≤6× ..... ***C. clavata***
5. Vesicles globose, subglobose, ovoid or slightly angular; sporophore branches rare or common; when present branches similar in size and rarely re-branching 6
6. Sporophores infrequent, poorly branched; sporangiola brownish to dark brown ..... ***C. phaeospora***
6. Sporophores common, well branched; sporangiola grayish to brownish ..... ***C. elegans***

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A comparative SEM study of morphological characters in *Cribraria*

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Abstract—*Cribraria* spp. were examined by SEM to determine detailed morphological features of the spore, nodes, and calyculus and to identify and describe the characters and character states for each microstructure examined. The character states defined include six for spore ornamentation (warted, crested, spiny, subreticulate, banded-reticulate, grooved-reticulate), three for node types (rounded, enlarged, irregular), and four for the upper calyculus margin (ribbed, irregular, toothed, entire). Our SEM studies show that interpretations of spore ornamentation character states based solely on LM may be incorrect and that SEM observations are crucial for discovering new characters and interpreting taxonomic morphological features.

Key words—*Cribrariaceae*, morphology, myxomycetes, plasmodial granules, slime molds

Introduction

Myxomycetes (now formally classified by www.indexfungorum.org in *Myxogastrea*) are a small, relatively homogeneous group of eukaryotic organisms with small fructifications (sporangia) and a spore size range of 5–15 µm (Stephenson & Stempen 1994). The ornamented spore surface is very difficult to assess using only light microscopy (LM) (Singer et al. 2006), and the magnification and resolution limitations of LM prevent close examination

of the fine structure of sporangia (Hatano 1985). Lane (1985) proposed that scanning electron microscope (SEM) techniques be used to describe new characters and to assist in the proper interpretation and taxonomic use of previously unknown features.

Spore ornamentation, size, and (in some species) the way in which the dehydrated spore wall collapses are among the most useful diagnostic characters for identifying myxomycete species. The ability of SEM to produce taxonomically significant information and to resolve minute details has proved superior to other forms of microscopy in visualizing important diagnostic characters (Schoknecht & Small 1972, Lyon & Kramer 1977). Although Gaither & Collins (1984) credited Schoknecht & Small as the first to emphasize SEM for resolving taxonomical questions in myxomycete systematics, Keller & Reynolds (1971) had previously described a new species of *Perichaena* with the aid of the SEM images. Subsequently several other authors have used SEM techniques to identify new taxonomic characters (Ellis et al. 1973, Rammeloo 1974, Eliasson & Sunhede 1980, Nelson et al. 1982) and to provide more inclusive descriptions of new species (Keller & Reynolds 1971, Keller et al. 1975, Mulleavy 1977, Raub et al. 1979, Whitney & Olive 1983).

Cribraria species produce some of the smaller sporangia (up to 1 mm in *C. zonatispora*), which in some instances are detectable only when the substratum is observed microscopically at 50–100× or when the specimens are produced in moist chamber cultures. Lado (2017) currently recognizes 46 species of *Cribraria*. Morphological characters that vary among species—type of nodes, surface spore ornamentation, calyculus margin, and nature of the peridial net—are among those most frequently used in taxonomic descriptions. In the literature, most species descriptions are based on LM observations; to date only 18 *Cribraria* species have been described with SEM details (Hatano 1985, Keller et al. 1988, Lado et al. 1999, Estrada-Torres et al. 2001, Moreno et al. 2001, Hatano 2002, Liu & Chang 2007, Hooff 2009). Hatano (1985), in a study of myxomycetes from Japan, provided SEM details for ten species in the *Cribrariaceae* (*Cribraria argillacea, C. aurantiaca, C. cancellata, C. cribrarioides, C. intricata, C. languescens, C. microcarpa, C. minutissima, C. rufa,* and *C. tenella*), although the spores were collapsed in *C. minutissima* and *C. rufa;* Keller et al. (1988), who discussed the systematics of *C. minutissima* and *C. confusa,* included SEM photographs with detailed spore morphology and noted that the spores tended to collapse along preformed lines over the surface. Lado et al. (1999) described *C. zonatispora* as having 'unique' spores, while Estrada-Torres et al. (2001) described unique spore ornamentation for their new species,

C. fragilis, from México. In their contribution to the myxomycetes of Baja California (México), Moreno et al. (2001) described the spore ornamentation as observed under SEM for *C. intricata, C. purpurea,* and *C. violacea*. Hatano (2002) studied and described the surface ornamentation of the sporangium, calyculus, peridial surface net and spore ornamentation for five species from the Korean Peninsula (*C. intricata, C. microcarpa, C. rufa, C. splendens* and *C. tenella*). More recently, Liu & Chang (2007) described *C. angulospora* and Hooff (2009) described *C. tecta* using characters observed under SEM.

In a general spore classification scheme for all myxomycetes, Hatano (1985) proposed seven types of spore ornamentation, including *Cribraria* in type I (warts) with four subtypes (a, b, c, d). Each subtype was defined based on the frequency or distribution of warts on the spore surface. Although he considered warts the predominant type of ornamentation in *Cribraria* spores, a completely different surface ornamentation has been observed under SEM in some *Cribraria* species, e.g. *C. zonatispora*, *C. fragilis*, and, *C. tecta* (Lado et al. 1999, Estrada-Torres et al. 2001, Hooff 2009).

In view of the morphological variability of the spore surface and other taxonomically important structures found in *Cribraria*, the objective of our study was to contribute to knowledge of the genus through SEM examination and to identify potentially informative morphological characters and character states of the microstructures that will eventually help reveal phylogenetic relationships within the genus.

Materials & methods

Taxon sampling

We obtained specimens representing 35 (of 46 known) *Cribraria* species from the U.S. National Fungus Collection, Beltsville MD, U.S.A. (BPI), the herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS, now donated to herb TLXM), and the myxomycete collection at the herbarium of the Universidad Autónoma de Tlaxcala, México (TLXM). Whenever more than two specimens per species were obtained, we processed the most abundant and typical (based on our comparison with the descriptions) collection for SEM observation. Here we describe only SEM observations for 21 species, as we were unsuccessful in processing the specimens representing the other 14 species.

Processing of the sporangium for SEM

Because material collected in the field is generally dehydrated when preserved in a herbarium, preparation for SEM observation required rehydration of the samples for eight hours in a 1:1 solution of concentrated ammonium hydroxide and distilled water. After rehydration, samples were dehydrated in a graded

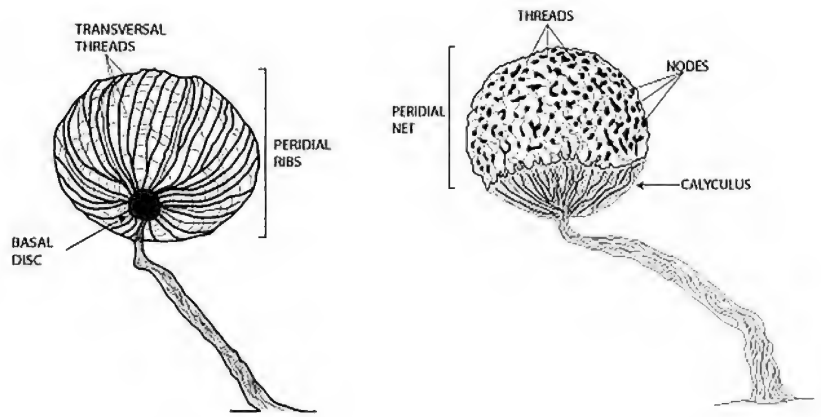

FIGURE 1. Morphological sporocarp structures described for *Cribraria* species.

ethyl alcohol series of 10, 20, 30, 50, 90 and 100% concentrations and then acetone solution for ten minutes at each step. The samples were then dried using a critical point technique, mounted on specimen stubs with double face adhesive tape, and coated with gold/ palladium in a high-vacuum chamber with a JFC-1100 Jeol Fine Coat Ion Sputter. The samples were examined with a JEOL JSM-5600 scanning electron microscope.

Results

The characters targeted under the SEM include (1) the peridium, (2) the type of node, (3) the margin and outer surface of the calyculus, and (4) the spore surface. Below, we detail the taxonomic characters for *Cribraria* as observed under SEM and briefly compare them with the characters described by previous authors (based on SEM and LM). For a better understanding of the morphological terms used in this paper, we also provide a drawing depicting important generic features in *Cribraria* (FIG. 1) and a brief glossary (p. 419).

Species & SEM descriptions

Cribraria atrofusca G.W. Martin & Lovejoy,
J. Wash. Acad. Sci. 22: 92, 1932. PLATE 1a–d

SPECIMEN BPI 828412—PERIDIUM: consisting of an upper net and a shallow cup at the base of the sporotheca. NODES: scarcely thickened, mostly elongated, connected by broad threads, sometimes with 2–3 free ends and forming angular isodiametric meshes. PLASMODIAL GRANULES: concentrated principally in

the nodes but also in the inner side of the threads. Calyculus: membranous, shallow, usually well developed, about one third the total height of the entire sporotheca, the margin bearing fine long tooth-like projections, the outer calyculus surface characterized by pronounced concentric folds. Spores: (SEM images slightly collapsed) surface ornamentation apparently composed of a reticulum formed by thick ribs, with 4–5 meshes on one hemisphere and with warts, sometimes fused and forming ridges, in the inner areas formed by the meshes of the reticulum.

Comments: The microstructure under SEM has not been previously reported for *C. atrofusca*. Martin & Alexopoulos (1969) described the spore ornamentation as "finely verrucose with faint, broken reticulate pattern" as seen by LM. Although most spores were collapsed in our study, we did observe that a ribbed-reticulate pattern and warted spore surface occur in this species. The concentric folds in the calyculus and the peridial net are the most distinctive features of this species.

Cribraria aurantiaca Schrad. Nov. Gen. Pl.: 5. 1797. Plate 1e–h

Specimen TNS 2612—Peridium: partially fugacious, consisting of a net occupying most of the sporotheca and leaving a calyculus in the base. Nodes: isodiametric, most rounded, thickened, connected by threads (c. 2 µm diam.) forming a regular mesh. Plasmodial granules: (c. 2 µm diam.) concentrated in the nodes. Calyculus: membranous, shallow, usually well developed, about one-third the total height of sporotheca, margin characterized by ribs connecting with peridial net, upper surface with delicate concentric folds, radial folds at the base of the calyculus. Spores: globose, 5–6 µm diam. with a surface ornamentation formed by warts; frequently several fuse to form an irregular ridge.

Comments: A SEM study by Hatano (1985) reported *C. aurantiaca* with a spore ornamentation composed of many warts, sometimes adhering to each other and forming irregular ridges. Our observations of the spore ornamentation, node, and calyculus characters agree with Hatano.

Cribraria cancellata (Batsch) Nann.-Bremek., Nederlandse Myxomyceten 92. 1975. Plate 2a–c

Specimen TLXM Rodríguez-Palma 2191—Peridium: partly evanescent, remaining as 50–60 ribs connected by fine transverse threads. Plasmodial granules: distributed along the rib surfaces, 1–2 µm diam. Spores: globose, c. 4–5 µm diam.; surface ornamentation composed of winding crests, sometimes

branched and forming a subreticulate pattern. Stalk: grooved and attenuate at the apex. Sporotheca: oblate, sometimes globose, usually umbilicate at the apex, generally nodding, rarely erect.

Comments: Martin & Alexopoulos (1969) and Lado & Pando (1997) described the spore surface as smooth or minutely punctate, but irregular and winding crests are easily seen with SEM. Hatano (1985), who described the spore ornamentation in the same way, referred to *C. cancellata* as having vein-like warts. We did not see a basal disc or calyculus in our specimen but did observe that the ribs extend from the stalk apex.

Cribraria costata Dhillon & Nann.-Bremek.,
Proc. Kon. Ned. Akad. Wetensch., C 81: 141. 1978. Plate 2d–h

Specimen TNS 16898—Peridium: partially evanescent, remaining as ribs that arise from the base to almost the top of the sporotheca, connected by perpendicular threads, forming a net at the upper part of the sporangium. Nodes: pulvinate, isodiametric, connected by strip-like threads that form an open mesh and with few free ends. Plasmodial granules: concentrated in the nodes. Calyculus: (if present) a basal disc. Spores: globose, 6–7 µm diam.; surface ornamentation composed of winding crestae, sometimes branched and forming a subreticulate pattern.

Comments: The specimen we studied fits well the original description given by Dhillon & Nannenga-Bremekamp (1978). The microstructure as seen by SEM has never been reported before for *C. costata*. The ribs radiating from the apex of the stalk and the pyriform shape of the sporotheca are the distinctive characters for this species.

Cribraria dictyospora G.W. Martin & Lovejoy,
J. Wash. Acad. Sci. 22: 91. 1932. Plate 3a–d

Specimen TNS 2936—Peridium: with a shallow basal cup and a fine meshed net in the upper portion of the sporotheca. Nodes: flattened and elongated, with narrow connecting threads with few free ends. Calyculus: shallow, occupying one-third the total height of the sporotheca, marked with radial folds on the outer surface, margin toothed. Spores: polyhedric, 5–6 µm diam., surface ornamentation composed of a wide meshed ribbed reticulum, giving a polyhedric appearance of the spores, with 4–5 meshes per spore side and warts in the inner surface of the meshes. Stalk: erect and striate. Sporotheca: globose or subglobose, erect to slightly nodding.

Comments: Although the spores seem to be slightly collapsed, the surface spore ornamentation is clearly formed by a reticulate pattern with fine warts inside the meshed areas. These are the first SEM observations of *C. dictyospora*.

Cribraria intricata Schrad. Nov. Gen. Pl. 7. 1797. Plate 3e–h

Specimen BPI 828726)—Peridium: partially evanescent, in most of the sporotheca remaining as a net but sometimes also as a basal disc or cup. Nodes: thickened, elongated, roughened, connected by ≤8 slender threads with two or three free ends. Plasmodial granules: concentrated on the outer side of the nodes. Calyculus: shallow when present, with the upper margin extending into ribs, which are connected with the peridial net; surface with prominent radial folds marked by the concentration of the plasmodial granules and fine concentric folds. When the calyculus is absent, subparallel ribs emerge from the stalk apex. Spores: globose, 4–5 µm diam., with the surface ornamentation formed by warts; frequently several fused to form an irregular ridge.

Comments: Hatano (1985, 2002) mentions that under SEM the spore surface of *C. intricata* has many warts with corrugate swellings. Although in our specimen most spores were collapsed, our SEM observations appear closer to those described by Hatano in 1985; our specimen showed a perfectly developed calyculus, contrasting with the observation by Hatano in 2002 in which the calyculus was reduced to a basal disc and the ribs emerged from the base of the sporotheca. As Martin & Alexopoulos (1969) stated, *C. intricata* is very variable in these features.

Cribraria languescens Rex,
Proc. Acad. Nat. Sci. Philadelphia 43: 394. 1891 Plate 4a–c

Specimen TLXM ET44090—Peridium: partially fugacious, remaining as a calyculus at the base and as a net in the upper portion. Nodes: pulvinate, isodiametric, connected by 5–6 threads that form an irregular open mesh with few free ends. Plasmodial granules: concentrated on the nodes. Calyculus: shallow, up to half of the sporotheca, with nearly even or somewhat toothed margins, with faint radial folds. Spores: globose, 6–7 µm diam., with the surface ornamentation formed by remarkably dense, 0.1–0.2 µm diam. warts, several frequently fused to form irregular and sometimes branched ridges. Stalk: long, slender, slightly grooved.

Comments: The surface ornamentation of the spore was described as densely warted, with the warts forming irregular ridges (Hatano 1985). Our study corroborates the observations of *C. languescens* by this author.

Cribraria laxa Hagelst. Mycologia 21: 298. 1929. PLATE 4d–h

SPECIMEN BPI 828894—PERIDIUM: partially evanescent, consisting of ribs that radiate from the stalk apex, a membranous calyculus at the sporothecal base, and a very lax net in the upper portion. NODES: isodiametric, sometimes elongated, pulvinate, connected by 4–5 slender threads, with 1–2 free ends, basal ribs with not connecting threads between them. CALYCULUS: irregular, shallow, formed by thin membrane between the basal ribs, with an irregular margin. SPORES: globose, 5–6 µm diam., with the surface ornamentation formed by 0.3–0.5 µm long spines, frequently fused to form irregular ridges in a sub-reticulate pattern.

COMMENTS: The spiny ornamentation of the spore in *C. laxa* forms a subreticulate pattern, seemingly unique in *Cribraria*. Although Martin & Alexopoulos (1969) described a net with elongated and large nodes, we also frequently observed rounded nodes.

Cribraria lepida Meyl, Bull. Soc. Vaud. Sci. Nat. 56:326. 1927. PLATE 5a–c

SPECIMEN BPI 828797—PERIDIUM: partially evanescent, remaining as a prominent and deep basal calyculus and an open net at the upper portion of the sporotheca. NODES: irregular, pulvinate, densely covered with plasmodial granules, connected by 4–5 thin threads, without free ends. CALYCULUS: deep, up to half of the sporotheca, margin even, outer surface granulose due to the presence of the plasmodial granules. SPORES: globose, 6–7 µm diam., with the surface ornamentation formed by warts that frequently fuse to form irregular short ridges. SPOROTHECA: globose or cylindrical, nodding.

COMMENTS: The spores of *C. lepida* have been described as smooth using LM (Martin & Alexopoulos 1969), but they are clearly warted with SEM. Although this species is similar to *C. violacea* in color, size, habit, and even the warted spore ornamentation, the nodes differ considerably: flattened in *C. violacea* and pulvinate in *C. lepida*. As seen with SEM, in *C. lepida* the nodes resemble a cluster of grapes due to the aggregation of plasmodial granules.

Cribraria macrocarpa Schrad. Nov. Gen. Pl.: 8. 1797. PLATE 5d–h

SPECIMEN BPI 828967)—PERIDIUM: partially fugacious, remaining as a shallow calyculus at the base and a dense net with very close meshes in the upper part. NODES: irregular, large and elongated, flattened, connected by 4–5 wide threads with membranous margins forming a close-meshed net, with 2–3 free ends. PLASMODIAL GRANULES: 2 µm diam., smooth, distributed on

the nodes and on the whole calyculus. Calyculus: occupying one-third the sporotheca, well developed, margin irregular with many perforations in the upper portion, with radial folds on outer surface. Spores: globose, 6–7 µm diam., with the surface ornamentation formed by warts, frequently fused to form irregular short ridges. Stalk: wide and slightly grooved. Sporotheca: globose or ovate, nodding or erect.

Comments: The calyculus with the perforated margin and the flattened and irregular nodes confirm the identity of *C. macrocarpa,* based on the LM description by Martin & Alexopoulos (1969). Adamonyte & Eliasson (2010) compared it within a species complex comprising *C. martinii, C. argillacea,* and *C. ferruginea*, which all share a perforated peridial calyculus. Nonetheless, we have no SEM evidence confirming this similarity of *C. macrocarpa* with the other species.

Cribraria microcarpa (Schrad.) Pers., Syn. Meth. Fun.:190. 1801. Plate 6a–d

Specimen BPI 828978—Peridium: partially fugacious, remaining as ribs at the base of the sporotheca and a net above; basal ribs not interconnected with fine threads, emerging from the apex of the stalk. Nodes: small, pulvinate, hemispherical or sometimes elongated, studded with plasmodial granules, connected by faint threads that form a close net with polygonal meshes and without free ends. Calyculus: absent. Spores: globose, 6–7 µm diam., with the surface ornamentation formed by 0.1 µm diam. spines. Sporotheca: globose, erect or nodding. Stalk: slender, the apex thinner than the rest of the stalk, plicate.

Comments: Hatano (1985) cited two types of spore ornamentation in *C. microcarpa*—one with warts and the other with spines. Our observations agree with Hatano's second choice, even though our spores were not completely round. Martin & Alexopoulos (1969) and Hatano (1985) observed that when present, the calyculus is reduced to a minute basal disc. Our SEM images clearly show that the basal disc is lacking but that ribs, which radiate from the apex of the stalk and connect with the peridial net, are present. Nannenga-Bremekamp (1993) described this feature as "threads springing from the stalk."

Cribraria mirabilis (Rostaf.) Massee, Monogr. Myxogastr.: 60. 1892. Plate 6e–h

Specimen TLXM GF44—Peridium: partially evanescent, remaining as sub-parallel ribs from the base to the middle of the sporotheca, connected by delicate threads and an irregular net in the upper portion. Nodes: flattened,

irregular, elongated, connected by 4–5 threads (similar in width to the basal ribs) with no free ends. Plasmodial granules: distributed along the ribs. Calyculus: not observed. Spores: globose, 5–6 µm diam., surface ornamentation composed of winding crests, sometimes branched to form a subreticulate pattern. Sporotheca: globose, erect or nodding.

Comments: *Cribraria mirabilis* has never previously been published based on SEM observation. SEM observation revealed that the spore ornamentation is composed of minute crests that sometimes fuse to give the appearance of ridges. Our SEM images also show that the ribs extend from the base of the sporotheca to form a well-developed net in the upper part.

Cribraria oregana H.C. Gilbert, Amer. J. Bot. 19:142. 1932. Plate 7a–c

Specimen TLXM HC183—Peridium: partially evanescent, remaining as a basal shallow calyculus and an irregular net in the upper portion. Nodes: flattened, irregular, connected by 4–5 threads, which form irregular meshes and open net with few free ends. Calyculus: shallow, with irregular margins and smooth surface. Spores: globose, 7–8 µm diam., with the surface ornamentation formed by minute and dense warts, frequently fused to form irregular short ridges. Sporotheca: globose, usually erect. Stalk: slender, slightly attenuated at the apex, longitudinally striate.

Comments: This is the first SEM description for *C. oregana*. Relying on LM, Martin & Alexopoulos (1969) described the spore ornamentation as "minutely warted, with a delicate reticulum" while Lado & Pando (1997) defined it as "spinulose." Our SEM examination revealed the absence of both reticulum and spines and the presence of abundant minute warts that fuse to form irregular short ridges.

Cribraria piriformis Schrad. Nov. Gen. Pl.: 4. 1797. Plate 7d–h

Specimen BPI 829249—Peridium: partially fugacious, remaining as a calyculus at the base of the sporotheca and dense net in the upper portion. Nodes: isodiametric to elongated, pulvinate, depressed on the inner side, covered with plasmodial granules, connected by 3–7 diam. threads that form a closed net and with 3–6 rounded free ends and wider tips. Calyculus: shallow, with irregular margins and with some rib-like projections connecting the peridial net and with pronounced concentric folds on the outer surface. Spores: globose, 6–7 µm diam., with the surface

ornamented by abundant minute warts in aggregates. Sporotheca: globose to pyriform, erect. Stalk: furrowed, slightly attenuated at the apex.

Comments: Ours is the first SEM description for *C. piriformis*. It has thickened nodes as well as wide connecting threads in the peridial net. Nodes on the inner side are markedly hollow (Pl. 7f). Under LM some authors (Martin & Alexopoulos 1969, Lado & Pando 1997, Adamonyte & Eliasson 2010) have described the spore ornamentation as densely warted, and our SEM images confirm this character.

Cribraria purpurea Schrad. Nov. Gen. Pl.: 8. 1797. Plate 8a–d

Specimen TLXM HC1869—Peridium: partially evanescent, remaining as a shallow basal calyculus and a net in the upper portion. Nodes: flattened, irregular in size and form, sometimes undifferentiated, connected by 3–6 very fine threads, with few free ends. Plasmodial granules: hollow, on the inner side of nodes, 2–3 µm diam., smooth. Calyculus: shallow, with irregular or toothed margin, with concentric folds. Spores: globose, 5–6 µm diam., with surface ornamentation composed of winding crests, sometimes branched and forming a subreticulate pattern. Sporotheca: globose, nodding.

Comments: *Cribraria purpurea* was described using SEM by Moreno et al. (2001). However, our examination revealed a different spore ornamentation composed of crests fused to form a sub-reticulate pattern, not observed in the image provided by Moreno et al. (2001, Fig. 21), who described the ornamentation as "baculate" (a completely different pattern). It would be interesting to confirm that both examined specimens represent *C. purpurea*.

Cribraria rubiginosa Fr. Syst. Mycol. 3:172. 1829. Plate 8e–h

Specimen TLXM ET2720—Peridium: partially fugacious, remaining as a deep basal cup and a loose net occupying the upper half of the sporotheca. Nodes: irregular, flattened, somewhat angular, filled with plasmodial granules and connected with strip-like threads, without free ends. Plasmodial granules c. 1–2 µm diam. Calyculus: deep, with irregular margins, radial folds covered with plasmodial granules at least at the base of the sporotheca. Spores: globose, 6–7 µm diam., ornamented by a prominent reticulum formed by grooves, with four meshes per side, and pronounced warts in the meshes, sometimes fused. Sporotheca: cylindrical, erect. Stalk: grooved.

Comments: Martin & Alexopoulos (1969) describe the spores as angular and with a warted ornamentation by LM. Our SEM image shows a very well defined

reticulum formed by grooves that cross the whole spore surface and warts between the meshes of the reticulum, in a similar way found on the spores of *C. angulospora*, a species recently described from Taiwan (Liu & Chang 2007). Nevertheless we suspect that *C. angulospora* may represent a synonym of *C. rubiginosa*. Martin & Alexopoulos (1969) mentioned that the angular shape of the spore depends on the angle in which the spore is observed in the microscope, but it is clear that this angularity is due to the presence of the reticulum.

Cribraria rufa (Roth) Rostaf. Śluzowce monogr.: 232. 1875. PLATE 9a–d

SPECIMEN BPI 829339—PERIDIUM: partially evanescent, remaining as a shallow basal calyculus and an open-meshed net in the upper portion of the sporotheca. NODES: flattened, irregular to elongate connected by 3–5 wide threads, without free ends. CALYCULUS: short, irregular margin, radially plicate ribs, extending from the base of the sporotheca and connecting with the peridial net, outer surface with very marked radial folds. SPORES: globose, somewhat angular, 7–8 µm diam., surface ornamentation composed of an open reticulum formed by thick ribs with warts in the inner areas formed by the meshes of the reticulum, the warts sometimes fused and forming short ridges. SPOROTHECA: globose or ovate, erect. STALK: erect, longitudinally striate.

COMMENTS: In a SEM study of *Cribrariaceae* from Korea, Hatano (2002) reported the spore ornamentation in *C. rufa* as a "corrugate swelling [that] connects each other and makes a vein-like protuberance." Our SEM observation of the spore surface shows a lax reticulum divided by spaces with warts included. Under LM Nannenga-Bremekamp (1991) described the spore ornamentation as having warts often connected by rows of smaller warts into a lax reticulum. Our observations agree with the description of spore ornamentation given by Nannenga-Bremekamp (1991). The angular appearance of the spore, as mentioned by Martin & Alexopoulos (1969), may be due to the presence of a lax reticulum on the spore surface.

Cribraria splendens (Schrad.) Pers., Syn. Meth. Fung.:191. 1801. PLATE 9e–h

SPECIMEN TLXM GF1769—PERIDIUM: partially fugacious, remaining as 4–5 thick ribs at the base and an open net in upper portion. NODES: flattened, not well differentiated, occasionally elongated, without free ends and connected by 3–4 fine threads, which have membranous margins and form an irregular and open meshed net; ribs radiating from the stalk and connecting with the peridial net. CALYCULUS: absent. SPORES: globose, 5–6 µm diam., with the

surface ornamentation formed by warts, sometimes fused to form irregular short ridges. Sporotheca: globose, nodding. Stalk: slender, curved at the apex, longitudinally striate.

Comments: Hatano (2002) described the spore ornamentation for *C. splendens* under SEM as "corrugate swellings" that sometimes are fused and then a vein-like protuberance are observed. Our SEM and his photographs agree, but we prefer to use the more appropriate term "warts" for this type of ornamentation, noting that sometimes the warts fuse to form irregular short ridges.

Cribraria tenella Schrad. Nov. Gen. Pl.: 6. 1797. Plate 10a–d

Specimen TLXM ET4832—Peridium: partially fugacious, remaining in the basal part of the sporotheca as a calyculus, and as a net in the rest. Nodes: pulvinate, rounded, few free ends, connected usually by four slender threads. Net irregular, meshes open, with wide connecting threads. Calyculus: shallow, the margin irregular, surface smooth, sometimes radial folds emerging from the base, marked by the presence of plasmodial granules. Spores: globose, minutely warted, the rest of the surface smooth, 5–6 μm diam. Sporotheca: globose, nodding.

Comments: Hatano (2002) reported the spore surface "with protuberances" for this *C. tenella*. In general the peridial net and spore descriptions by Hatano (1985; 2002) correspond our results, except for the calyculus, which seem more regular and shallow in Hatano's description and more irregular and deeper in our SEM plate.

Cribraria violacea Rex, Proc. Acad. Nat. Sci. Philadelphia 43:393. 1891. Plate 10e–h

Specimen BPI 829575—Peridium: partially evanescent, remaining as a deep calyculus at the base and as a net in the upper portion. Nodes: flattened, irregular in size and form, with hollow protuberances due to the plasmodial granules, connected by delicate threads, which form an open net, with few and irregular meshes, without free ends. Calyculus: deep, occupying half or two thirds of the sporotheca, margin toothed, with the surface studded with plasmodial granules. Spores: globose, 6–7 μm diam., with the surface ornamentation formed by warts, sometimes fused forming irregular short crests. Sporotheca: globose or cylindrical, erect. Stalk: slender, grooved.

Comments: Moreno et al. (2001) are the only authors to have described this species as "baculate with small crests" using SEM. The two SEM images

are similar, but our SEM plate shows warts that are much closer and form irregular short crests. Under LM most authors have described *C. violacea* as minutely warted (Martin & Alexopoulos 1969, Nannenga-Bremekamp 1991, Lado & Pando 1997).

Cribraria vulgaris Schrad. Nov. Gen. Pl.: 6. 1797. PLATE 11

SPECIMEN BPI 829689—PERIDIUM: partially fugacious, remaining as a calyculus at the base and as a net in the upper portion of the sporotheca. NODES: irregular or isodiametric, flattened, connected by 4–5 threads that form an irregular net without free ends. CALYCULUS: occupying about one third of the lower portion of the sporotheca, with irregular margins, forming spiny teeth that give rise to the net, the surface marked by delicate radial folds. SPORES: 5–6 µm diam., the surface ornamented by warts that sometimes fuse to form irregular short ridges (not possible to confirm in collapsed spores). SPOROTHECA: globose, nodding. STALK: erect, slightly attenuate at the apex, longitudinally striate.

COMMENTS: Martin & Alexopoulos (1969) described the spore ornamentation of *C. vulgaris* based on LM as "minutely punctate;" nevertheless Nannenga-Bremekamp (1991), Lado & Pando (1997), and Adamonyte & Eliasson (2010) described it as "slightly angular, warted with faint ridges or with wartlets forming a lax reticulum." Warts are present and distributed on the spore surface in our SEM photographs, but the presumed reticulum was not observed due to the collapsed spore.

Character coding under phylogenetic perspective

Character description plays such an essential role in the phylogenetic inference process, determination of characters and character states is one of the most fundamental steps in any taxonomic work (Kirchoff 2001). Newton & De Luna (1999) noted, "we need not ascertain a priori if a character or its states have a common origin, or if character states are homologous" because these result from the cladistics analysis. The final result from the inference cladistics process must serve as the foundation for any discussion about evolutionary character change that will allow us to corroborate or change the homology hypothesis based on strict cladistic principles (Wirkner & Richter 2010).

In a phylogenetic context, Sereno (2007) defined the logical basis for morphological characters, where he concluded that "characters are simply features expressed as independent variables and character states

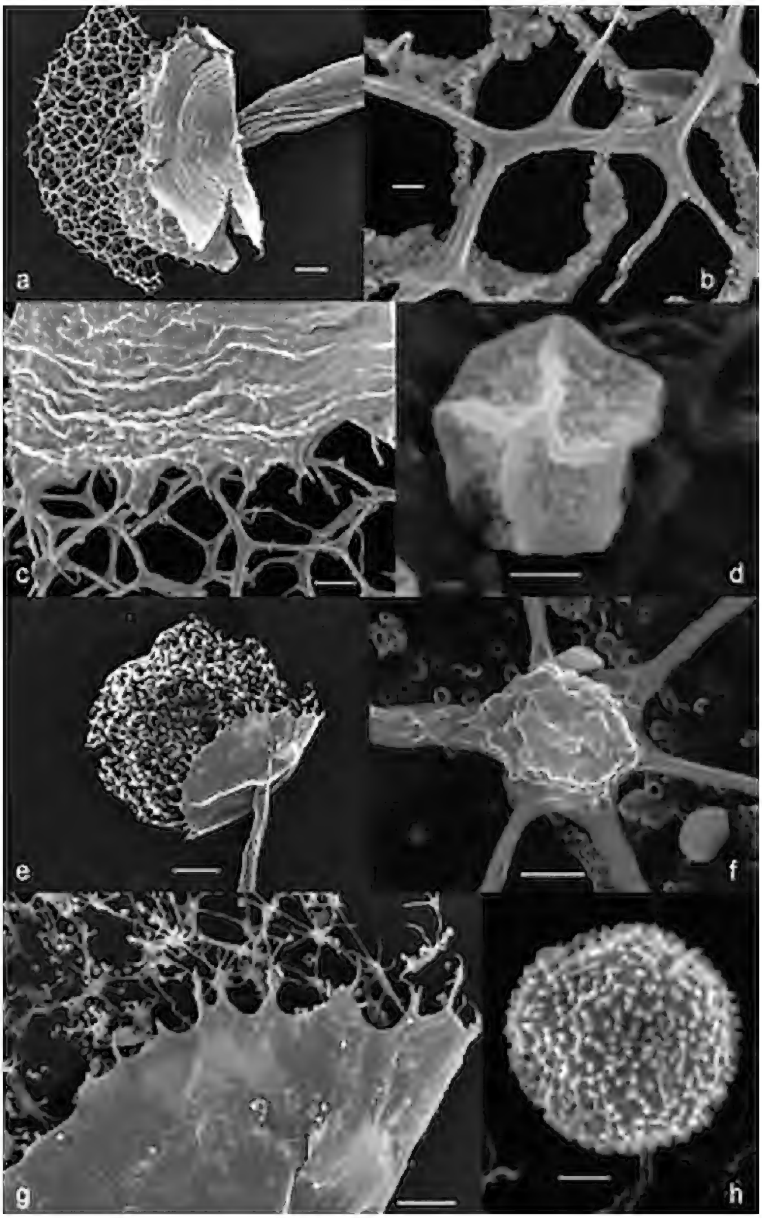

Plate 1. ***Cribraria atrofusca***. a. Sporocarp; b. Enlarged node with plasmodic granules; c. Margin and outer surface of calyculus; d. Collapsed spore. ***Cribraria aurantiaca***. e. Sporocarp; f. Node; g. Margin and outer surface of calyculus; h. Surface spore ornamentation. Scale bars: a, e = 100 μm; b = 10 μm; c = 20 μm; d = 2 μm; f = 5 μm; g = 50 μm; h = 1 μm.

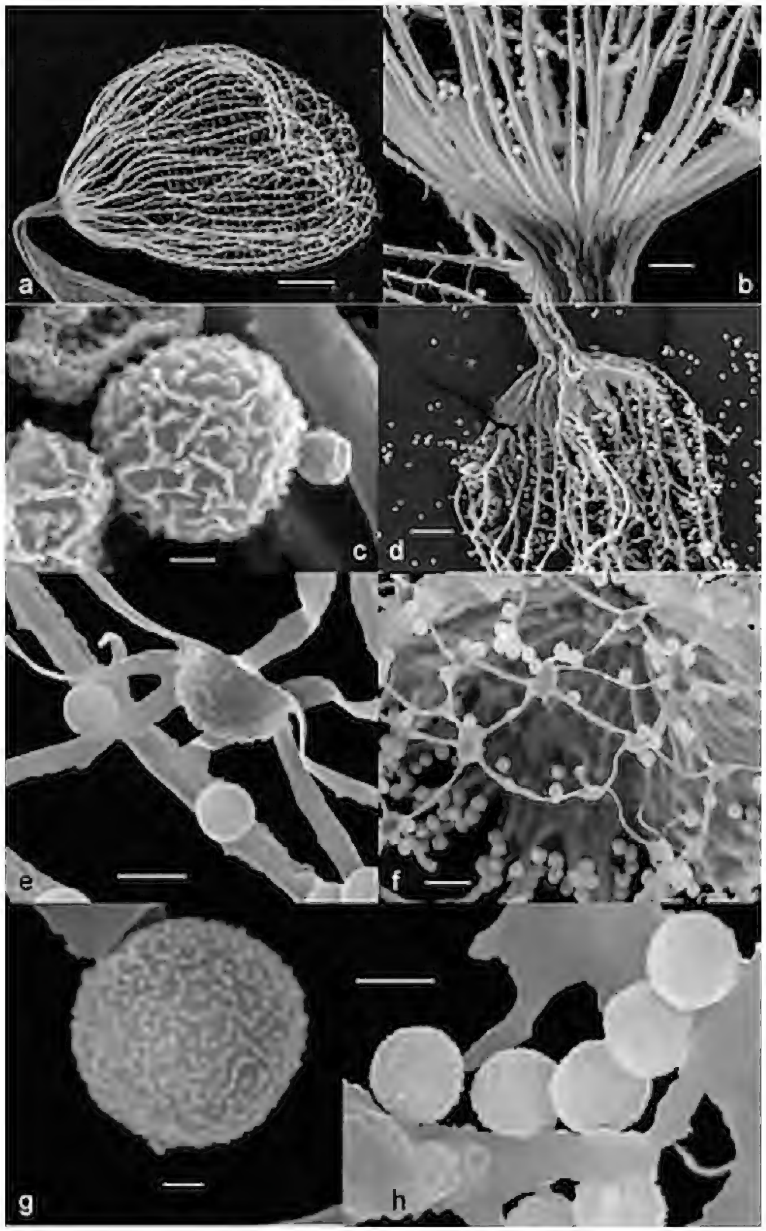

PLATE 2. ***Cribraria cancellata***. a. Sporocarp; b. Peridial ribs emerging from the stalk apex; c. Spore and plasmodic granule. ***Cribraria costata***. d. Sporocarp; e. Node of peridial net; f. Peridial net without free ends; g. Spore surface ornamentation with crestae; h. Spores. Scale bars: a = 100 µm; b, f = 20 µm; c, g = 1 µm; d = 50 µm; e =10 µm; h = 5 µm.

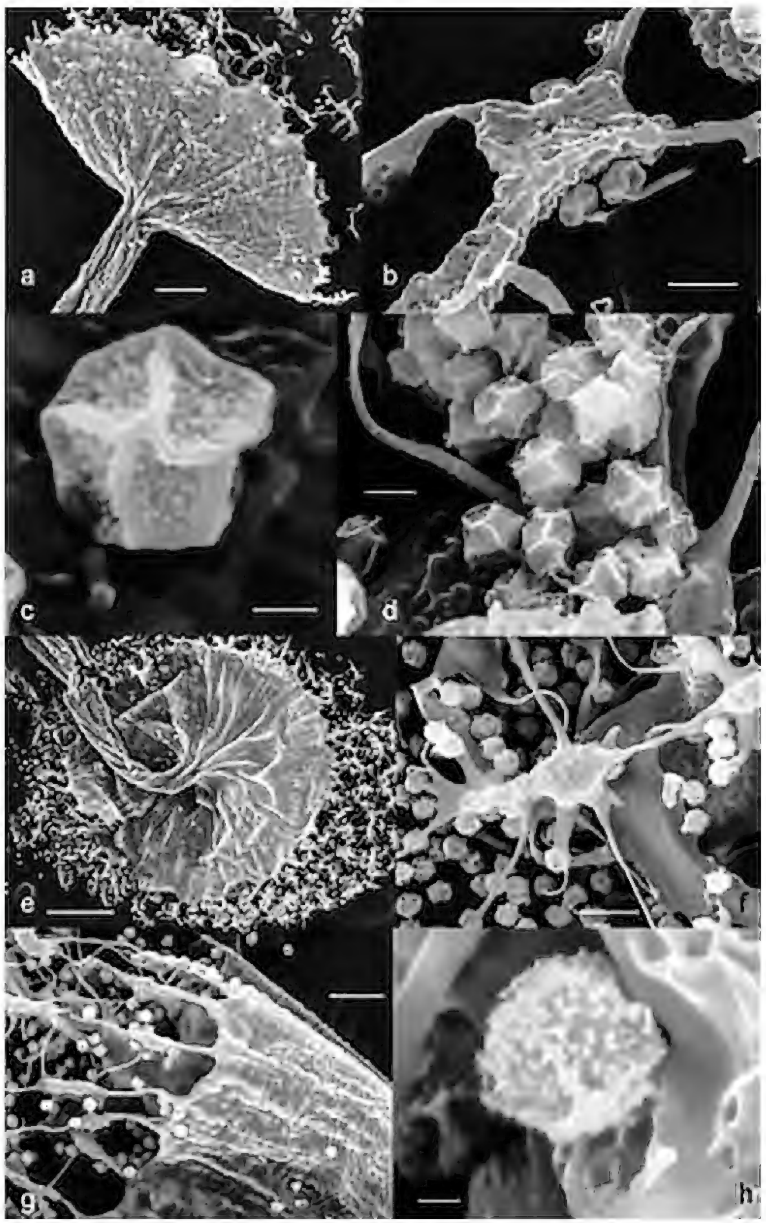

PLATE 3. ***Cribraria dictyospora***. a. Margin details and outer surface of calyculus; b. Node; c. Collapsed spore with reticulum; d. Collapsed spores showing surface reticula. ***Cribraria intricata***. e. Sporocarp; f. Peridial node with 2–3 free ends; g. Calyculus margin connecting with the peridial net and details of the outer surface; h. Spore surface ornamentation with warts. Scale bars: a = 50 µm; b, f = 10 µm; c= 2 µm; d = 5 µm; e = 100 µm; g = 20 µm; h = 1 µm.

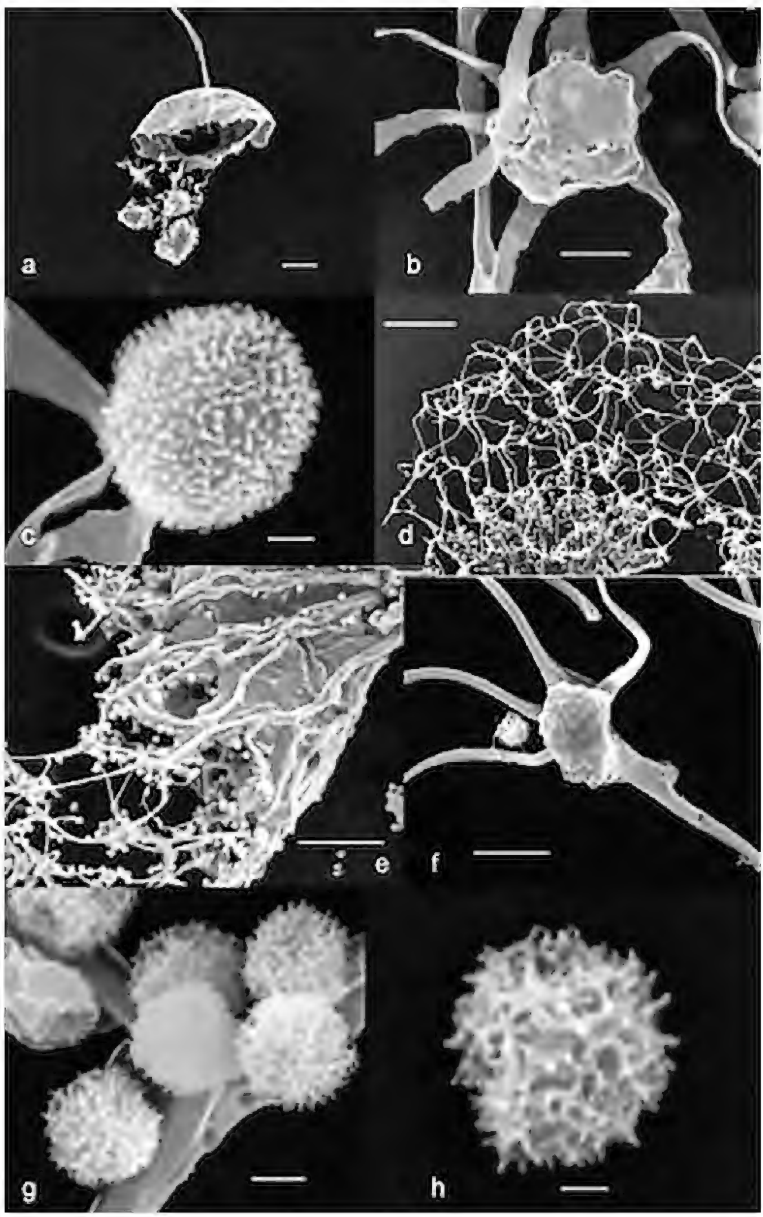

Plate 4. ***Cribraria languescens***. a. Sporocarp; b. Peridial node apparently without free ends; c. Spore surface ornamentation with warts. ***Cribraria laxa***. d. Peridial net; e. Peridial ribs emerging from the base of the sporocarp and details of the calyculus surface; 50 µm. f. Thickened node; g. Spores; h. Spore ornamentation. Scale bars: a, e = 50 µm; b = 5 µm; c, h = 1 µm; d = 100 µm; f = 10 µm; g = 2 µm.

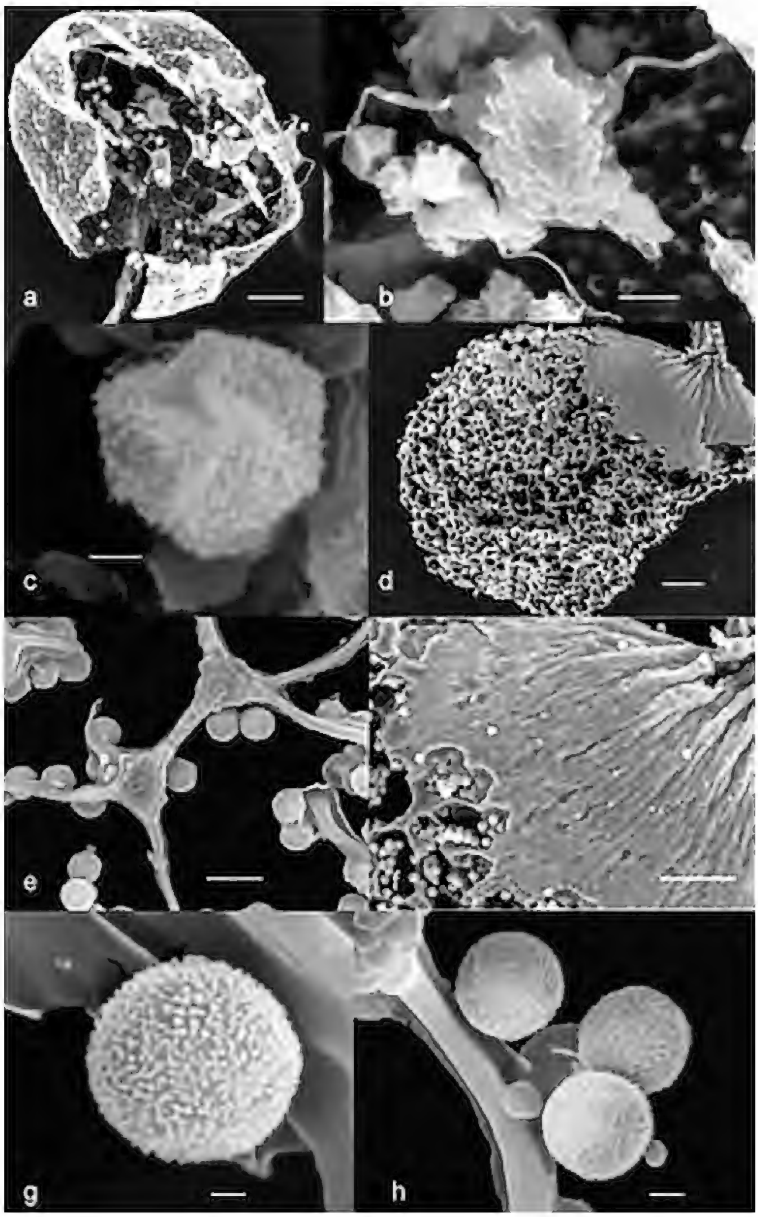

Plate 5. ***Cribraria lepida***. a. Sporocarp; b. Peridial node; c. Spore. ***Cribraria macrocarpa***. d. Sporocarp showing the peridial net and calyculus; e. Peridial node with plasmodic granules and spores; f. Details of calyculus outer surface; g. Spore surface ornamentation; h. Spores and plasmodic granules. Scale bar: a = 20 µm; b = 5 µm; c, g = 1 µm; d = 100 µm; e = 10 µm; f = 50 µm; h = 2 µm.

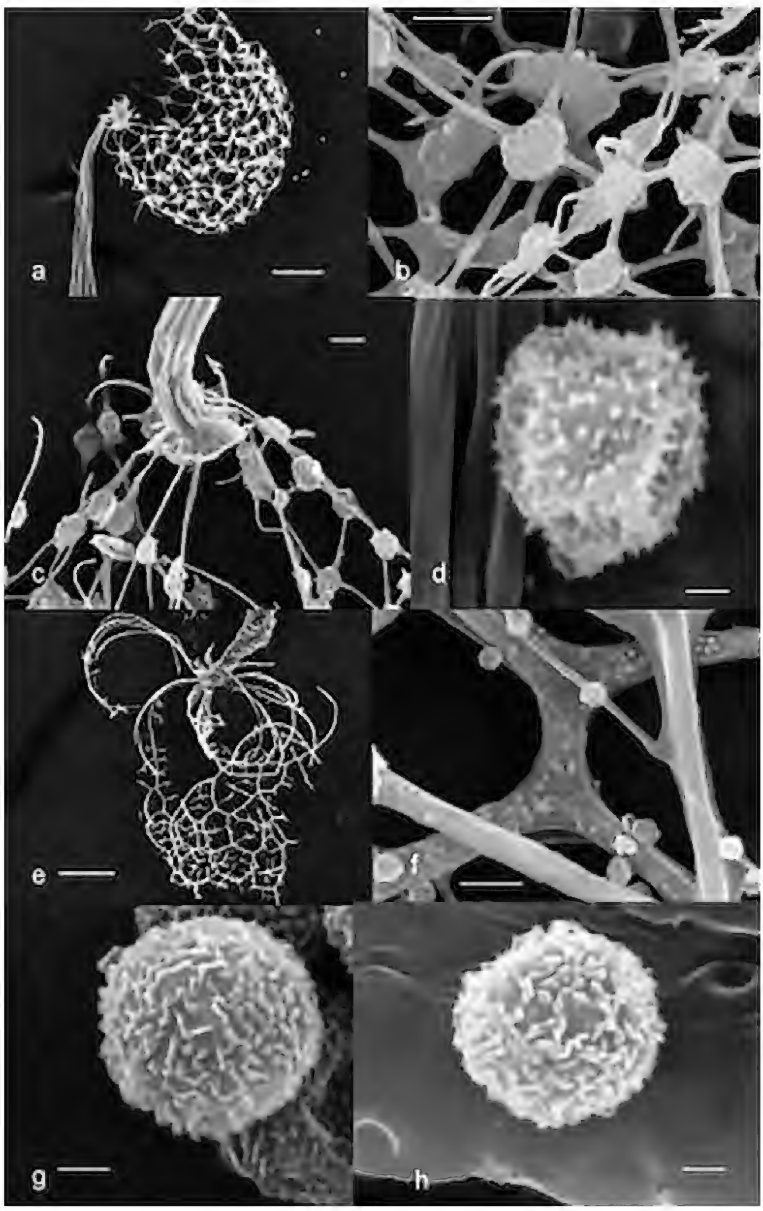

PLATE 6. ***Cribraria microcarpa***. a. Peridial net of the sporocarp; b. Peridial nodes with plasmodic granules; c. Peridial ribs emerging from the apex of the stalk; d. Spore with spines as surface ornamentation. ***Cribraria mirabilis***. e. Sporocarp showing the peridial ribs and net; Peridial net without well-defined nodes; g. Spore surface ornamentation; h. Spore. Scale bars: a = 50 μm; b, c, f = 10 μm; d, g, h = 1 μm; e = 200 μm.

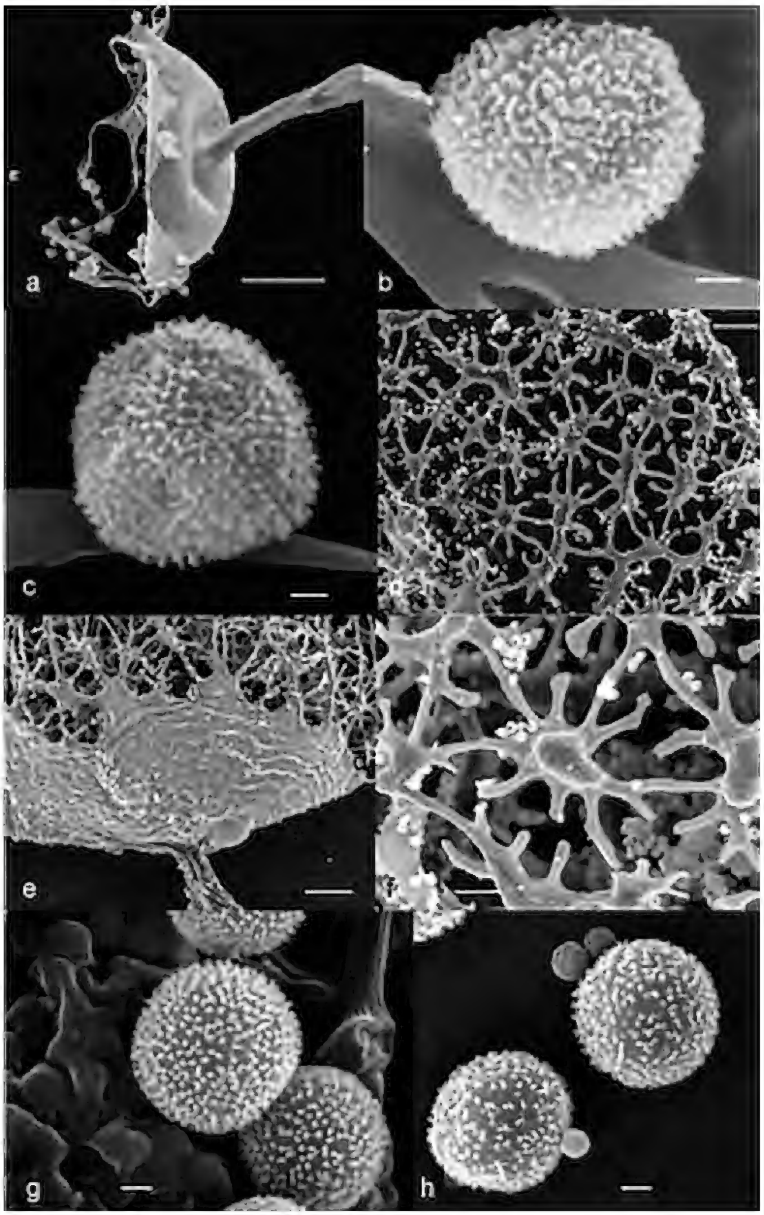

Plate 7. ***Cribraria oregana***. a. Sporocarp; b. Spore surface ornamentation; c. Spore with warts. ***Cribraria piriformis***. d. Peridial net and spores; e. Outer surface of calyculus and margin connecting with the peridial net; f. Peridial node with 2–3 free ends; g. Spores; h. Spores and plasmodic granules. Scale bars: a, d = 50 µm; b, c, g, h = 1 µm; e = 100 µm; f = 20 µm.

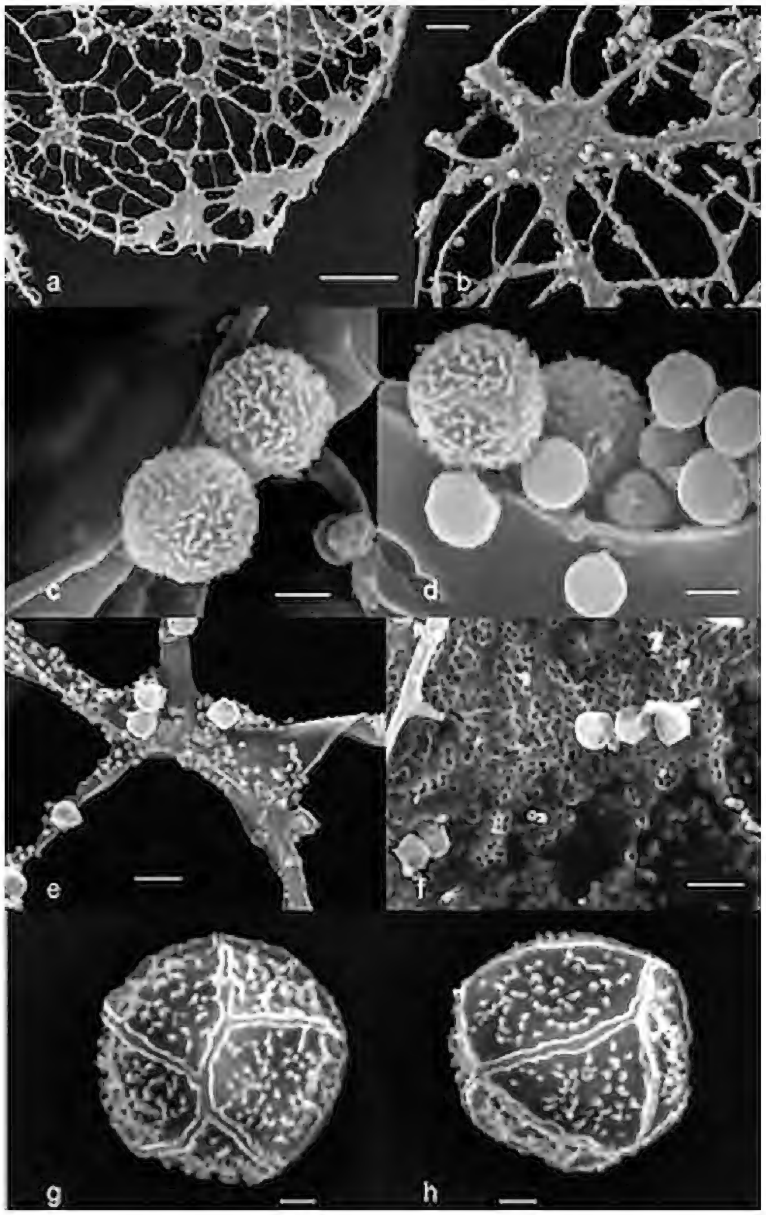

Plate 8. ***Cribraria purpurea***. a. Peridial net; b. Nodes flattened without free ends; c. Spores with surface ornamentation composed of crests; d. Spores and plasmodic granules. ***Cribraria rubiginosa***. e. Flattened node with spores and plasmodic granules; f. Inner surface details of calyculus; g. Spore details of surface ornamentation showing warts and a reticulum groove crossing the spore; h. Spore seen in angular view. Scale bars: a =100 µm; b = 20 µm; c, d = 2 µm; e, f = 10 µm; g, h = 1 µm.

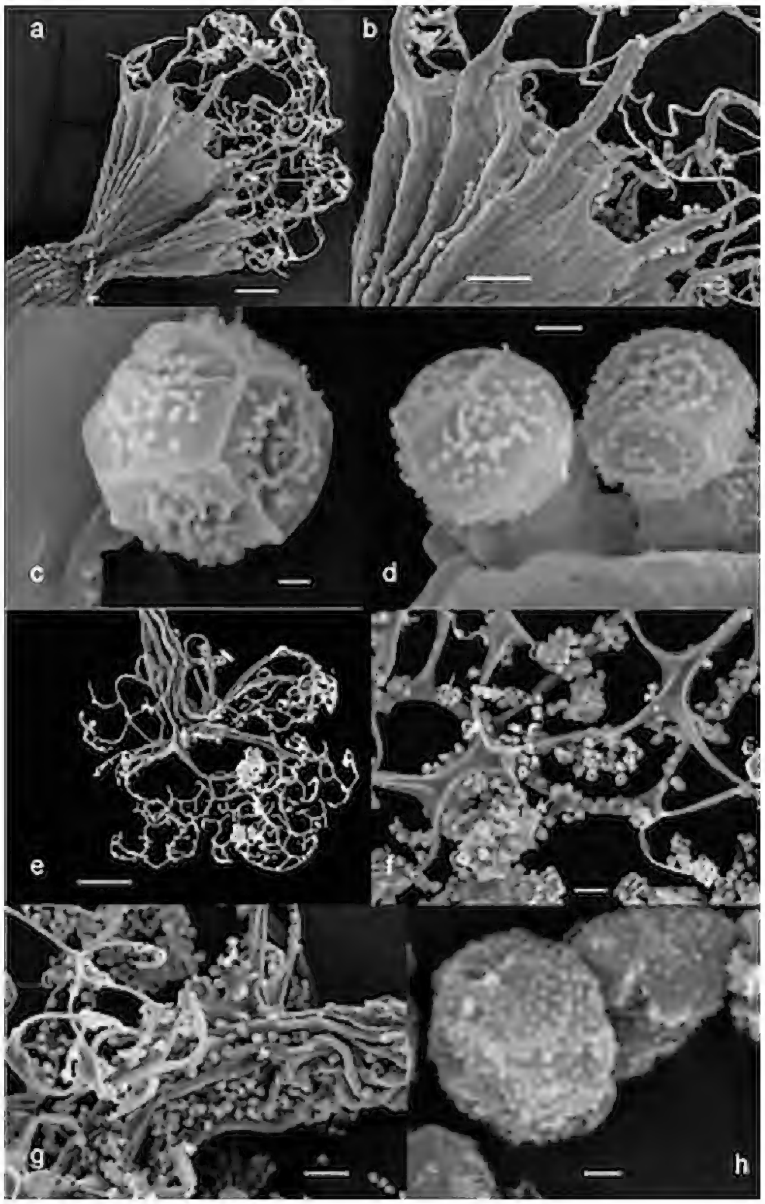

Plate 9. ***Cribraria rufa***. a. Sporocarp; b. Peridial ribs extending from the base of the sporotheca; c. Spore showing a reticulum and warts in the inner area; d. Spores. ***Cribraria splendens***. e. Sporocarp; f. Node flattened and elongated; g. Peridial ribs extending from the apex of the stalk; h. Spore surface ornamentation with warts. Scale bars: a, e =100 μm; b = 50 μm; c, h = 1 μm; d = 2 μm; f, g = 20 μm.

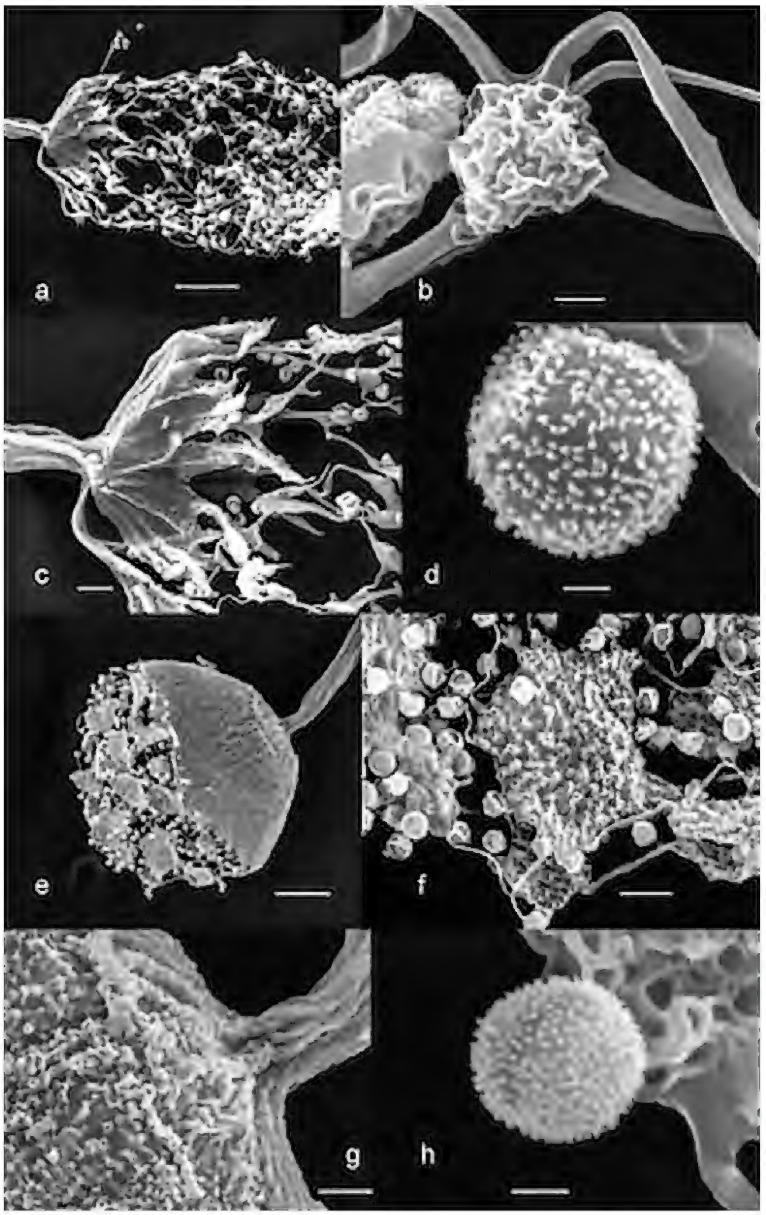

Plate 10. ***Cribraria tenella***. a. Sporocarp; b. Pulvinate and rounded node; c. Calyculus with radial folds emerging from the base; d. Spore surface ornamentation with warts. ***Cribraria violacea***. e. Sporocarp; f. Peridial node with hollow protuberances caused by plasmodic granules; g. Calyculus surface (detail); h. Spore with surface ornamentation formed by warts. Scale bars: a, e =50 μm; b, h = 2 μm; c, f, g = 10 μm; d = 1 μm.

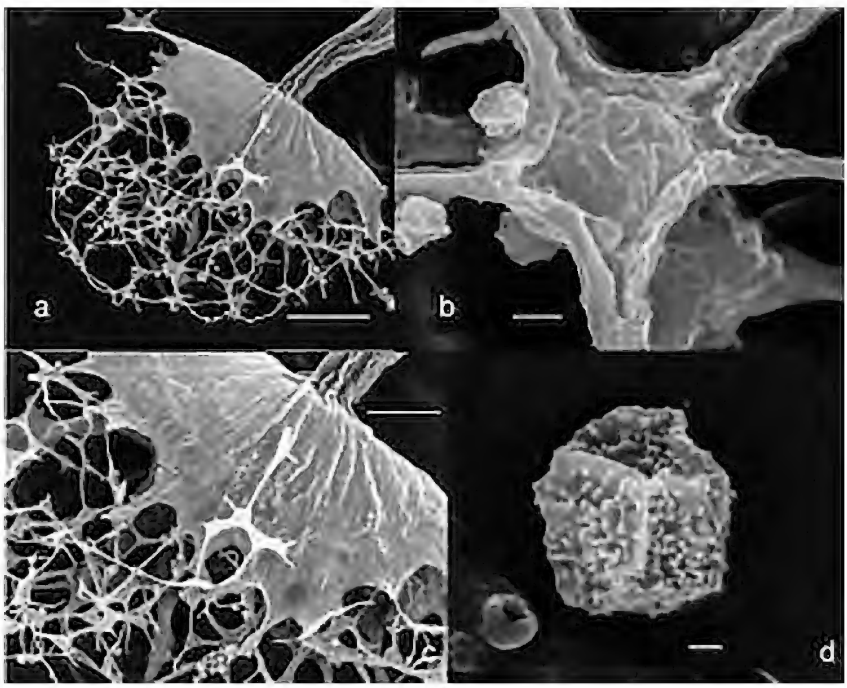

Plate 11. ***Cribraria vulgaris***. a. Sporocarp; b. Peridial node flattened and without free ends; c. Outer surface of calyculus and margin connecting with the peridial net; d. Collapsed spore with warts as spore surface ornamentation. Scale bars: a = 100 µm; b = 5 µm; c = 50 µm; d = 1 µm.

the mutually exclusive conditions of that character." Four components for character statements were recognized: locators, variable, variable qualifiers, and characters states. All of this information can be expressed in symbolic form (for more details see Sereno 2007: Table 4).

The rationale for defining character and character states (character conceptualization), therefore, is a crucial step in evolutionary morphology. In order to render the information obtained with the use of SEM for the genus *Cribraria*, we have applied the scheme proposed by Sereno (2007) for morphological characters with a symbolic connotation. Here we summarize the data obtained for the specimens analyzed whose phylogenetic information could be tested through a cladistics framework. These results were interpreted as suggesting that a character has at least two different character states (variables feature), and considering all the range variation observed for the species included in the present study (see Table 1, p. 416).

Table 1. Symbolic notation for characters and character states in sporotheca and spores of the *Cribraria* species.

Character (locator)	Character states (variables)	Symbolic notation*
	Sporotheca	
Peridial net	(0) Absent; (1) present	L_1: V_0; V_1
Peridial ribs	(0) Absent; (1) present	L_1: V_0; V_1
Calyculus	(0) Absent; (1) present	L_1: V_0; V_1
Nodes differentiated	(0) Absent; (1) present	L_1: V_0; V_1
Thickness of nodes	(0) Pulvinate; (1) flattened	L_1: V_0; V_1
Type of nodes in peridium	(0) Enlarged; (1) isodiametric; (2) irregular	L_1, L_2: V_0; V_1; V_2
Calyculus upper margin	(0) Entire; (1) irregular; (2) ribbed; (3) Toothed	L_1, L_2: V_0; V_1; V_2; V_3
Calyculus surface	(0) Radial folds; (1) concentric folds	L_1, L_2: V_0; V_1
	Spore surface ornamentation	
Warts	(0) Absent; (1) present	L_1: V_0; V_1
Crests	(0) Absent; (1) present	L_1: V_0; V_1
Reticulum	(0) Absent; (1) present	L_1: V_0; V_1
Bands	(0) Absent; (1) present	L_1: V_0; V_1
Spiny-subreticulate	(0) Absent; (1) present	L_1: V_0; V_1
Grooved-reticulate	(0) Absent; (1) present	L_1: V_0; V_1

*L_1 = primary locator, L_2 = secondary locator; V_n = character state

Conclusions

In this paper, we have described spore ornamentation variables (character states) as revealed by SEM never before noted for species such as *C. ferruginea* and *C. laxa*. Studies of micro-morphological characters in *Cribraria* with SEM, such as spore ornamentation and the shape of nodes, demonstrate that interpretations of these character states based on LM alone may be incorrect or completely lacking. This is because LM does not make certain features prominent or even noticeable in the same way as SEM. *Cribraria*, as observed by Ing (1999) and Adamonyte & Eliasson (2010), is a challenging genus due to overlapping morphological variation among the various species, making it problematical to circumscribe particular features with accuracy. Therefore, SEM is a fundamental tool for taxonomic studies in this genus as well as for other myxomycete taxa for which similar empirical difficulties exist. Further SEM research in *Cribraria*, either of same species or those not included in this work, will certainly confirm or amplify the morphological variation noted here.

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Glossary

Basal disc: A small portion of the calyculus remaining at the base of the sporotheca.

Calyculus: The lower portion of the sporotheca remaining as a persistent structure like a cup (Keller & Braun 1999)

Nodes: "The thickened area at the point of juncture where several threads meet to form the peridial network in the upper part of the sporotheca" (Keller & Braun 1999).

Peridial net: A persistent peridium that remains as a reticulate structure in the sporotheca (from Martin & Alexopoulos 1969).

Peridial ribs: Subparallel elements of the peridium, interconnected by short transverse threads, which may arise from the base of the sporotheca or extend from the calyculus margin.

Plasmodial granules: Microscopic, usually dark-colored structures found in the sporotheca of *Cribraria*, on either the peridial nodes or calyculus. These have been called "dictydine granules" (Martin & Alexopoulos 1969; Keller & Braun 1999) or "lime globules" (Nannenga-Bremekamp 1991). We prefer to use the term "plasmodial granules" based on their plasmodial origin.

Spore ornamentation: The surface of the spore, as seen under light microscope, may be smooth or have variously formed projections. Ornamentation features include:

Bands: Flat (generally wide) regions that typically cross to form a reticulate pattern.

Crests: projections distributed in waves.

Grooved-reticulate: a net-like pattern (reticulum) formed by deep grooves or furrows.

Reticulum: the pattern produced by warts distributed in lines that cross in a net-wise fashion.

Warts: short obtuse projections distributed over the surface.

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New records of *Ascomycota* from tropical dry forest in Sonora, Mexico

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Abstract—Eight new records of ascomycetes from Sierra de Álamos-Río Cuchujaqui Biosphere Reserve in Sonora, Mexico are reported. *Hypoxylon anomalum* and *Munkovalsaria donacina* represent new records for Mexico, while *Bionectria byssicola*, *Fracchiaea heterogenea*, *Hypoxylon lenormandii*, *Lasiodiplodia theobromae*, *Peroneutypa scoparia*, and *Pseudocosmospora vilior* are new records for Sonora.

Key words—*Botryosphaeriales, Coronophorales, Hypocreales, Xylariales*

Introduction

The Sierra de Álamos-Río Cuchujaqui Biosphere Reserve (ACBR) presents an extraordinary biological richness, with more than 1200 plant species in an area of 92,899 ha. It is located in the south of Sonora, Mexico, preserving part of the North American tropical dry forest intermixed with xerophilous scrub, oak-pine forest, and riparian vegetation (SEMARNAT 2015). Its floral and faunal components have been studied by Robichaux & Yetman (2000).

Twenty-two ascomycetes been recorded previously from ACBR: *Anteaglonium abbreviatum* (Schwein.) Mugambi & Huhndorf [≡ *Glonium abbreviatum* (Schwein.) M.L. Lohman], *Biscogniauxia nummularia* (Bull.)

Kuntze, *Daldinia clavata* Henn., *D. concentrica* (Bolton) Ces. & De Not., *D. eschscholtzii* (Ehrenb.) Rehm, *H. ferrugineum* G.H. Otth [≡ *H. rubiginosum* var. *ferrugineum* (G.H. Otth) J.H. Mill.], *D. fissa* Lloyd, *D. loculata* (Lév.) Sacc., *Hypoxylon anthochroum* Berk. & Broome, *H. haematostroma* Mont., *H. polyporus* (Starbäck) Y.M. Ju & J.D. Rogers, *H. rubiginosum* (Pers.) Fr., *Hysterobrevium mori* (Schwein.) E. Boehm & C.L. Schoch [≡ *Hysterographium mori* (Schwein.) Rehm], *Oedohysterium insidens* (Schwein.) E. Boehm & C.L. Schoch, *Rhytidhysteron rufulum* (Spreng.) Speg., *Xylaria arbuscula* Sacc., *X. feejeensis* (Berk.) Fr., *X. microceras* (Mont.) Berk., *X. poitei* (Lév.) Fr., *X. striata* Pat., *X. striata* var. *sonorensis* F. San Martín et al., and *X. uniapiculata* F. San Martín & J.D. Rogers (Pérez-Silva et al. 1996; San Martín et al. 1999a,b; Méndez-Mayboca et al. 2010; Chacón et al. 2014). This study aims to continue the expansion of our knowledge of ascomycete diversity in the ACBR.

Materials & methods

The Sierra de Álamos-Río Cuchujaqui Biosphere Reserve was surveyed with two samplings in October of 2013 and 2014, in the localities of El Cajon (27°01′42″N 108°45′23″W, 373 m a.s.l.) and Rancho La Sierrita (26°58′25″N 108°56′42″W, 528 m a.s.l.). The collected specimens were described from fresh material, processed, and deposited in the Herbarium, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico (ENCB). The species were determined by observing cut sections of the ascomata in temporary preparations mounted in 70% alcohol, 10% KOH, and lactophenol with cotton blue (LB) and compared with identification keys (Aptroot 1995a,b; Ju et al. 2005; Burgess et al. 2006; Carmarán et al. 2006; Chaverri et al. 2011; Herrera et al. 2013). New records for Sonora are indicated by a single asterisk, and for Mexico with two asterisks.

Taxonomy

Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae

****Lasiodiplodia theobromae*** (Pat.) Griffon & Maubl.,
Bull. Soc. Mycol. France 25: 57 (1909) Figs 1, 2

Pycnidia of (360–)470–510(–540) × (210–)350–430(–500) µm, subglobose, obpyriform to pyriform, dark brown to black, immersed in the surface of the host, erumpent when mature. Paraphyses hyaline, cylindrical, septate, occasionally branched, with rounded tips of 45–55 × 3–4 µm. Conidiogenous cells, cylindrical, hyaline, thin and smooth wall. Conidia of (19–)21–31(–32.5) × (12–)13–15.5(–18.5) µm, subovoid to ovoid-ellipsoid, broadly rounded apices tapering or truncate at the base, initially hyaline and aseptate with a thick wall,

dark olive, dark brown to black, with a transverse septum and longitudinal grooves or sulci when mature.

Specimens examined: **MEXICO, Sonora:** El Cajón, 14 Oct 2013, T. Raymundo 4819, 4827; Rancho La Sierrita, 15 Oct 2013, T. Raymundo 4827, D. Castro-Bustos 164.

Habitat & distribution—Widely distributed in tropical and subtropical regions, *L. theobromae* grows gregariously on decaying fruit such as *Ceiba acuminata* (S. Watson) Rose, and Alves et al. (2008) indicate that it is plurivorous. In México, it has been reported from the states of Campeche, Colima, Distrito Federal, Estado de México, Guerrero, Guanajuato, Jalisco, Michoacán, Nayarit, Puebla, Querétaro, Tabasco, Tlaxcala, and Veracruz (Medel & Chacón 1988, Cibrián-Tovar et al. 2007). This is a first record for Sonora.

Comments—*Lasiodiplodia theobromae* is recognized by its black pycnidia, immersed and erumpent when mature, which produce subovoid and dark brown conidia with conspicuous longitudinal grooves. It is considered a virulent plant pathogen of hundreds of host species around the world. In Mexico, Cibrián-Tovar et al. (2007) consider it an opportunistic fungus that attacks stressed trees and produces various toxins that could favor leaf wilt in several tropical trees. A similar species, *L. pseudotheobromae* A.J.L. Phillips et al., differs by its dark brown and slightly larger (23.5–32 × 14–8 µm) conidia and occurrence in Costa Rica on *Acacia mangium*, *Citrus aurantium*, *Coffea* sp., and *Rosa* sp. (Alves et al. 2008). *Lasiodiplodia parva* A.J.L. Phillips et al. is also similar but produces smaller (16–23 × 10–13 µm) conidia and has been recorded only on *Theobroma cacao* in Colombia and Sri Lanka (Alves et al. 2008).

Dothideomycetes*, inc. sed., *Dacampiaceae

*****Munkovalsaria donacina*** (Niessl) Aptroot,
Nova Hedwigia 60: 346 (1995) Figs 3,4

Stroma inconspicuous, pseudothecia immersed, erumpent; solitary to gregarious, globose, (125–)200–250 µm diam., with a conspicuous ostiole. Peridium a textura angularis, 10–15 µm thick, with 3–6 hyaline layers on the inner side, golden brown on the outside; pseudoparenchyma formed by subglobose to angular cells 5–6.5 µm diam. Hamathecia with numerous pseudoparaphyses, 1–2.5 µm diam., with septa spaced at intervals of 10–20 µm, anastomosing, hyaline, inamyloid. Asci bitunicate, hyaline when young, 50–70 × 6–9 µm, cylindrical-clavate, with long stipes, octosporous, obliquely uniseriate to biseriate apically when mature. Ascospores 14–17 × 6–7.6 µm,

ellipsoidal to clavate, asymmetrical with a central septum that is transverse, constricted, and slightly submedian, brown yellowish and reddish brown with two green lipid droplets when mature, with slightly sharp ends; wall thin, slightly spinose, without gelatinous cover.

Specimen examined: **MEXICO, Sonora:** El Cajón, 14 Oct 2013, E. Escudero-Leyva 244b.

Habitat & distribution—Cosmopolitan and pantropical species. Gregarious, immersed in wood of woody angiosperms, where it coexists with *Peroneutypa scoparia*. *Munkovalsaria donacina* has been reported in a variety of hosts, mainly *Acanthaceae*, *Anacardiaceae*, *Fabaceae*, *Malvaceae*, *Moraceae*, *Juglandaceae*, *Rutaceae*, and *Verbenaceae*. Reported from Africa, Asia, Australasia, Europe, North America, and South America (Aptroot 1995b). This is the first record from México.

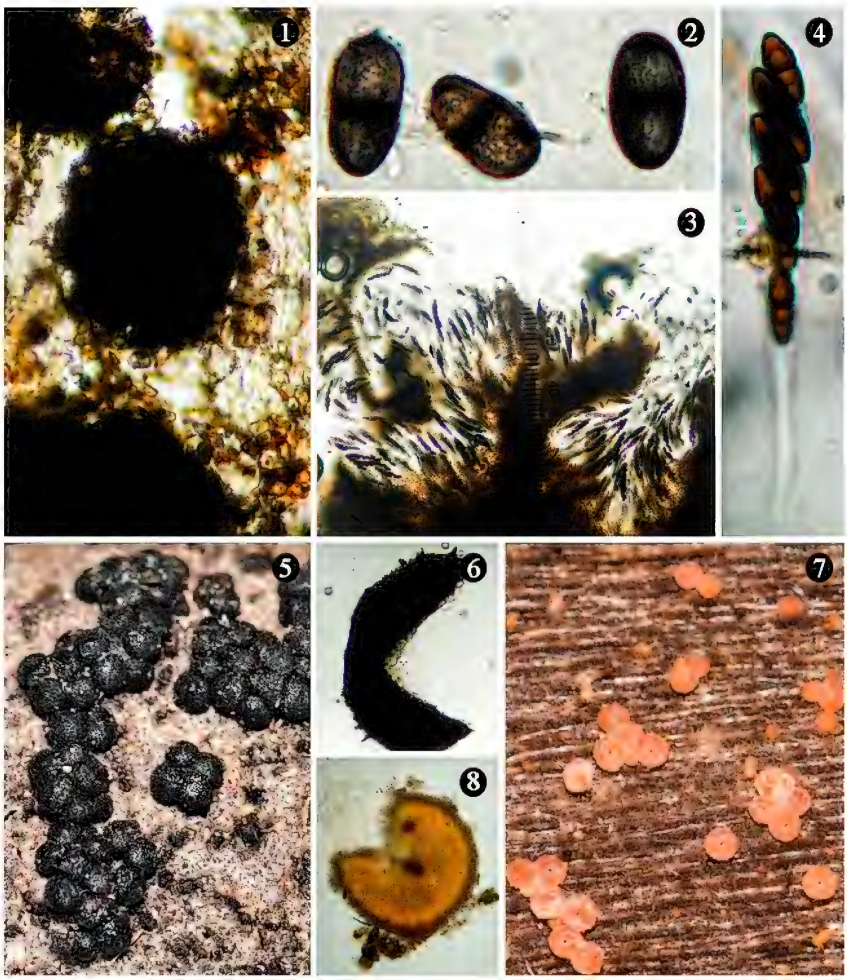
Comments—*Munkovalsaria donacina* is characterized by gregarious pseudothecia. A similar species, *M. appendiculata* Aptroot is distinguished by its ascospores (12–15 × 4–5 µm, fusiform with two hyaline polar appendages), growth on *Zea mays* in secondary vegetation, and distribution in Hong Kong (Aptroot 2004). *Didymosphaeria conoidea* Niessl is also similar but produces ascospores that are 9–12 × 4–6 µm, ellipsoidal to clavate, and with slightly sharp ends. *Munkovalsaria* is characterized by hamathecia with transversely septate pseudoparaphyses and euseptate ascospores, while *Didymosphaeria* produces hamathecia with narrow trabeculate paraphysoids and thinly distoseptate ascospores (Aptroot 1995b).

Sordariomycetes, Coronophorales, Nitschkiaceae

****Fracchiaea heterogenea*** Sacc.,

Atti. Soc. Veneto-Trentino Sci. Nat. 2: 163 (1873) Figs 5, 6

Perithecia gregarious in groups of 20–40, rarely solitary, erumpent, sessile, subglobose to globose, 500–700 µm diam., completely closed without ostiole, non-collapsing when dry, glossy black. Surface when young black verrucose with 18–25 µm long spines, becoming smooth when mature. Wall a textura angularis, with thick-walled pseudoparenchymatous cells with Munk pores. Quellkörper rudimentary. Paraphyses absent. Asci 80–100 × 15–18 µm, cylindrical, rounded at the apical end, with short stipes, multispored (≤200 ascospores), multiseriate, arranged obliquely in several longitudinal rows. Ascospores hyaline, 7.6–9 × 1.5–2 µm, cylindrical, slightly curved to allantoid, eventually with central septum and lipid droplets at the ends, walls thin and smooth.



Figs 1–8. ***Lasiodiplodia theobromae***. 1. Pycnidia. 2. Conidia. ***Munkovalsaria donacina***. 3. Pseudothecia. 4. Ascus and ascospores. ***Fracchiaea heterogenea***. 5. Perithecia in the substrate. 6. Perithecium in KOH. ***Bionectria byssicola***. 7. Perithecia in the substrate. 8. Perithecium in KOH.

Specimens examined: **MEXICO, Sonora:** Rancho La Sierrita, 15 Oct 2013, T. Raymundo 4848, R. Valenzuela 15262, D. Castro-Bustos 156.

Habitat & distribution—Cosmopolitan according to Nannfeldt (1975). *Fracchiaea heterogenea* grows on decaying branches of newly fallen trees, and is considered saprophytic and/or parasitic on other fungi. Chaverri et

al. (2011) recorded it for Costa Rica. In Mexico, it has been reported from Tamaulipas and Oaxaca (San Martín 1996, Raymundo et al. 2014). First record for Sonora.

Comments—The studied material matches the description by San Martín (1996), who reported *F. heterogenea* for the first time from Mexico in El Cielo Biosphere Reserve. Nannfeldt (1975) and San Martín (1996) considered it synonymous with *Nitschkia broomeana* (Berk.) Nannf., but *Nitschkia* species have octosporous asci, while *Fracchiaea* species have multisporous asci. We follow these criteria. The anamorph is not known yet.

Sordariomycetes, Hypocreales, Bionectriaceae

****Bionectria byssicola*** (Berk. & Broome) Schroers & Samuels, Z. Mykol. 63: 152 (1997) Figs 7, 8

Perithecia 290–400 µm diam., globose to subglobose, orange to pink when fresh, pale yellowish and laterally depressed when mature; no color change in KOH. Wall with whitish verrucae, 25–35 µm thick, dark red, textura epidermoidea, cells 10.4–12.8 × 8–12.8 µm. Asci 65–70 × 6–8.4 µm, clavate, with a visible apical ring in LB, hyaline, octosporous, uniseriate. Ascospores 13.6–15.2 × 4.8–5.6 µm, ellipsoidal to oblong, with a septum in the middle, hyaline, thin-walled, finely spinulose.

Specimen examined: **MEXICO, Sonora:** Rancho La Sierrita, 15 Oct 2013, R. Valenzuela 15272.

Habitat & distribution—*Bionectria byssicola,* which grows gregariously on trunks of newly fallen trees, has been cited from China, Taiwan, and Costa Rica (Nong & Zhuang 2005, Guu et al. 2010, Chaverri et al. 2011). In México it has been reported from the National Park Lagunas de Chacahua, Oaxaca (Raymundo et al. 2014). First record for the Sonoran mycobiota.

Comments—*Bionectria byssicola* is characterized by its orange to pink perithecia with whitish verrucae that change to pale yellowish in KOH. The anamorph [described as *Clonostachys byssicola* Schroers] forms sporodochia with curved conidia. Our Sonoran material matches the descriptions of Chinese and Taiwanese material (Chaverri et al. 2011, Guu et al. 2010). A similar species is *B. grammicospora* (Ferd. & Winge) Schroers & Samuels, which differs by its orange perithecia that do not change color in KOH, ascospores of 12.8–13.6 × 4.4–4.8 µm, and by the synnema in its anamorph [described as *Clonostachys grammicospora* Schroers & Samuels] (Schroers & Samuels 1997).

Sordariomycetes, Hypocreales, Nectriaceae

Pseudocosmospora vilior (Starbäck) C. Herrera & P. Chaverri, Mycologia 105: 1301 (2013) Figs 9, 10

Perithecia 209–248 × 171–199.5 µm, obpyriform, red changing to dark red in KOH, smooth, inconspicuous stroma. Neck 95–110.2 × 28.5–43.7 µm, occasional periphyses emerging from the ostiole (inconspicuous to the naked eye). Wall smooth, 20–40 µm thick, orange or yellowish in LB, textura epidermoidea; cells 8–12 × 2.4–4.8 µm. Asci 50.4–57.6 × 5.4–6.4 µm, cylindrical, without apparent apical apparatus, hyaline, octosporous, uniseriate. Ascospores 6.4–8.4 × 4–5.6 µm, ellipsoidal, constrained by a central septum, hyaline to yellowish, thin-walled, verrucose.

Specimen examined: **MEXICO, Sonora:** El Cajón, 14 Oct 2013, T. Raymundo 4808.

Habitat & distribution—*Pseudocosmospora vilior* grows gregariously on the surface of *Xylariaceae*. It has been recorded from China, Taiwan, and Costa Rica (Nong & Zhuang 2005, Guu et al. 2007, Chaverri et al. 2011). In México it has been reported from the National Park Lagunas de Chacahua, Oaxaca (Raymundo et al. 2014). It is cited for the first time for Sonora.

Comments—*Pseudocosmospora vilior* is characterized by smooth red perithecia that change to dark red with a white ostiole in KOH. Our Sonoran material matches the descriptions of Costa Rican and Taiwanese material (Chaverri et al. 2011, Guu et al. 2007). *Cosmospora flammea* (Tul. & C. Tul.) Rossman & Samuels is similar, but produces perithecia that emerge in dense clusters from wood or scale insects and ascospores of 16–20 × 7.5–10 µm (Herrera et al. 2013). *Macroconia gigas* (J. Luo & W.Y. Zhuang) Gräfenhan & Seifert is also similar, differing by its perithecia with yellowish trichomes and larger (13.5–23 × 5.5–7 µm) ascospores (Luo & Zhuang 2007).

Sordariomycetes, Xylariales, Diatrypaceae

Peroneutypa scoparia (Schwein.) Carmarán & A.I. Romero, Fungal Diversity 23: 84 (2006) Figs 11, 12

= *Peroneutypa heteracantha* (Sacc.) Berl., Icon. Fung. 3: 81 (1902)

Stromata 3–5 mm diam., pustuliform, black, erumpent, semicircular, with small bulges formed by 3–13 perithecia per stroma, monostichous or polystichous. Perithecia 400–600 µm diam., globose to ovoid, with thick black wall and surrounded by xylem tissue. Necks 800–1200 × 80–120 µm, extending conspicuously above the stroma, with cruciform ostiolar openings, surrounded by a velvety layer of yellowish to pale brown mycelia. Asci 10–18

× 4–5 µm, clavate, octosporous with amyloid apical ring. Ascospores 4–5 × 1–1.5 µm, pale yellow in KOH, greenish in mass, moderately allantoid, with two lipid droplets at the ends, aggregated to biseriate in the ascus.

Specimen examined: **MEXICO, Sonora:** Rancho La Sierrita, 15 Oct 2013, T. Raymundo 4857.

Habitat & distribution—This species grows gregariously on decaying debarked wood in tropical dry forests. Reported from Africa, Europe, Asia, North America, and South America (Rappaz 1987). In Mexico, *P. scoparia* has been reported from México City, Morelos, Nuevo León, Oaxaca, San Luis Potosí, and Tamaulipas (Pérez-Silva 1986, as *P. heteracantha*; Chacón 2003; Carmarán et al. 2006; Raymundo et al. 2014). This is the first record for Sonora.

Comments—*Peroneutypa scoparia* is characterized by pustuliform erumpent stromata and by perithecia with prominent and cruciform ostiolar necks at the apex. Our Sonoran material matches the description of Rappaz (1987). A similar species is *Eutypella portoricensis* (Petr.) Rappaz, with similar stromata, prominent but thin ostiolar necks, and larger (4–7 × 1.5–1.8 µm) ascospores (Rappaz 1987).

Sordariomycetes, Xylariales, Xylariaceae

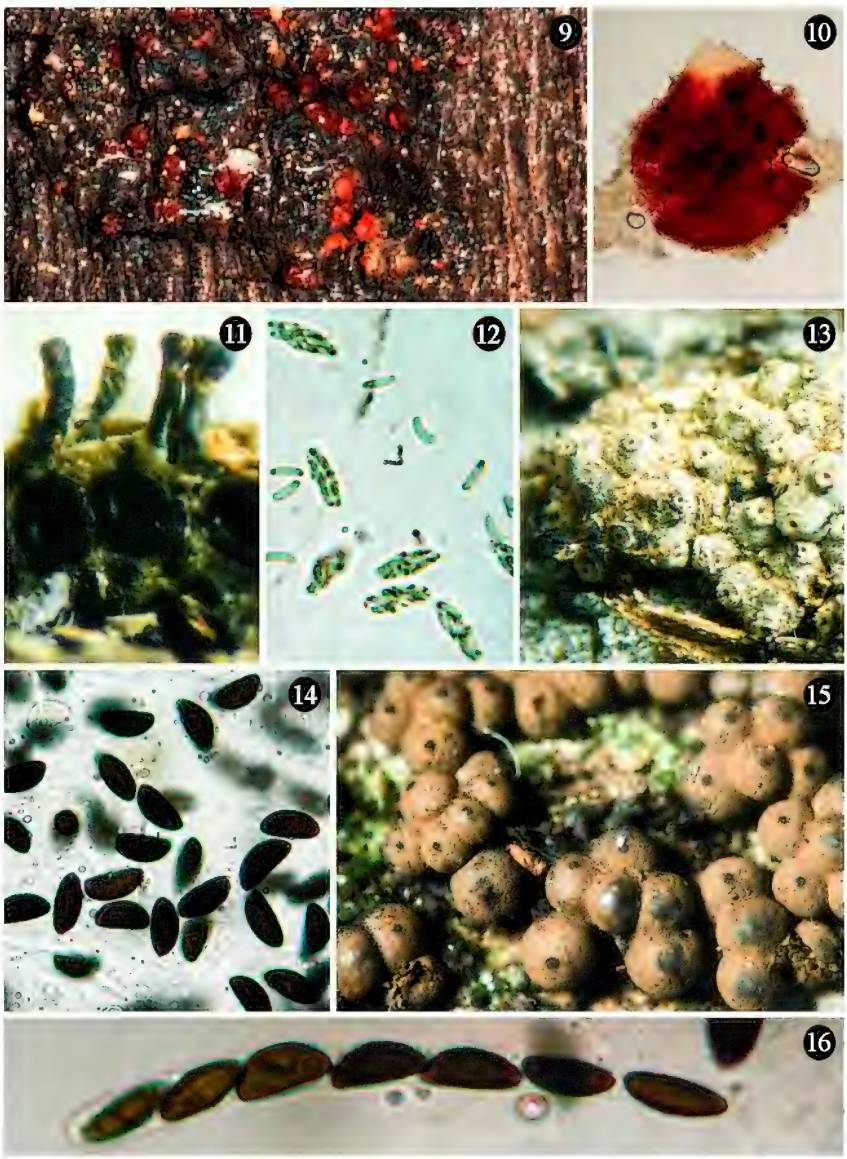
*****Hypoxylon anomalum*** J.D. Rogers, Y.M. Ju & Oses,
Mycologia 97: 562 (2005) Figs 13, 14

Stroma 4–6 mm diam., aggregated, with grouped perithecia, conspicuous. The tissue between the perithecia is not to slightly conspicuous, pale orange. Perithecia 400–600 µm diam., obovoid, orange to ocher brown and glossy dark brown, releasing pale yellowish pigments in KOH. Ostioles with umbilicate surface. Asci 80–98 × 10–12 µm, stipes 40–66 µm long, apical ring 1.4–2 × 3–4 µm, discoid, inamyloid, octosporous, uniseriate. Ascospores 12–15.5 × 6.5–8 µm, inequilaterally ellipsoid, with broadly rounded ends, dark brown to black; rectilinear germinal aperture with dehiscent perisporium and smooth episporium.

Specimens examined: **MEXICO, Sonora:** El Cajón, 14 Oct 2013, T. Raymundo 4809; Rancho La Sierrita, 15 Oct 2013, R. Valenzuela 15249.

Habitat & distribution—*Hypoxylon anomalum* grows gregariously on decaying branches. Reported in Costa Rica (Ju et al. 2005); it is recorded for the first time for México.

Comments—Our Mexican material matches the description of *H. anomalum* by Ju et al. (2005). A similar species, *Hypoxylon olliforme* San Martín et al., is



Figs 9–16. ***Pseudocosmospora vilior***. 9. Perithecia in *Hypoxylon*. 10. Perithecium in KOH. ***Peroneutypa scoparia***. 11. Stromata and perithecia. 12. Asci and ascospores. ***Hypoxylon anomalum***. 13. Stromata and perithecia. 14. Ascospores. ***Hypoxylon lenormandii***. 15. Stroma and perithecia. 16. Ascus and ascospores.

distinguished by its stroma that is effuse, pulvinate to rosellinoid, reddish brown and releasing reddish orange pigments in KOH, globose (200–300 µm diam.) perithecia, and ascospores that are 10.5–12 × 4.5–5 µm, dark brown, ellipsoidal (San Martín et al. 1999d).

****Hypoxylon lenormandii*** Berk. & M.A. Curtis,
J. Linn. Soc., Bot. 10: 385 (1868 ["1869"]) Figs 15, 16

Stroma 1500–10000 × 800–4000 × 1.5–2 mm, flat to effuse-pulvinate; perithecia not to slightly conspicuous, surface violet to violet brown with dark granules, sepia when mature, with removable yellowish green to olive pigments. The tissue between the perithecia is inconspicuous to slightly conspicuous, black, 200–700 µm thick. Perithecia 500–1000 × 300–400 µm, tubular, black, with ostioles below the stromal surface opening the stromal layer. Asci 72–98 × 4.5–6 µm, with 28–56 µm long stipes, apical ring of 1.4–2 × 3–4 µm, discoid, amyloid, octosporous, uniseriate. Ascospores 7–9 (–12) × 3.5–5.5 µm, ellipsoidal with broadly rounded ends, dark brown, rectilinear germinal aperture with indehiscent perisporium and smooth episporium.

Specimen examined: **MEXICO, Sonora: El Cajón**, 14 Oct 2013, D. Castro-Bustos 129, 144.

Habitat & distribution—*Hypoxylon lenormandii* grows gregariously on decaying branches and trunks from fallen trees. Recorded in United States (Vasilyeva et al. 2007) and México, where it has been reported in Campeche, Chiapas, Nuevo León, Oaxaca, Quintana Roo, and Tamaulipas (San Martín et al. 1999c; Raymundo et al. 2014). This is the first record for Sonora.

Comments—Our Sonoran material of *H. lenormandii* matches the description by San Martín et al. (1999c) for Mexican specimens; it is mostly consistent with the United States specimens, which have somewhat larger (10–15 × 4–6.5 µm) ascospores (Vasilyeva et al. 2007).

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Dictyotrichocladium aquaticum gen. & sp. nov. and *Minimelanolocus aquatilis* sp. nov. from freshwater in Brazil's semiarid region

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Abstract—A new genus and species, *Dictyotrichocladium aquaticum*, is described and illustrated from submerged decaying leaves of *Calophyllum brasiliense* in the Brazilian semiarid region; it is distinguished by macronematous, sometimes micronematous conidiophores, and monoblastic, terminal conidiogenous cells that produce solitary, long pyriform, cylindrical, long clavate, turbinate to vermiform, curved or sigmoid, dictyoseptate, black or dark pigmented conidia that usually do not secede but remain attached after maturation. A new species, *Minimelanolocus aquatilis*, characterized by obclavate, 3–5-euseptate, verruculose, pale brown conidia, is described and illustrated from decaying twigs of an unidentified plant in the Brazilian semiarid region.

Key words—aquatic fungi, Caatinga, taxonomy, tropics

Introduction

Freshwater hyphomycetes, which are essential for recycling complex organic matter in aquatic environments (Jones & Pang 2012), have part or entire life

cycles in water (Thomas 1996). Goh & Hyde (1996) delineated three freshwater groups: the Ingoldian fungi, the aero-aquatic hyphomycetes, and the facultative aquatic hyphomycetes. The present study focused on facultative aquatic hyphomycetes that include hyaline dematiaceous genera producing conidia that are mainly unmodified by aquatic environments (Goh & Hyde 1996, Shearer et al. 2007).

In Brazil, the first taxonomic research on facultative aquatic hyphomycetes occurred in the Brazilian semiarid region (Caatinga) and was followed by explorations in the Amazon and Atlantic Forest (Barbosa & Gusmão 2011; Barbosa et al. 2011, 2013; Silva & Gusmão 2013; Monteiro & Gusmão 2014; Moro et al. 2015; Fiuza et al. 2015a,b, 2016, 2017). These studies revealed a high biodiversity of facultative aquatic hyphomycetes and a large number of new taxa.

During studies on freshwater hyphomycetes on submerged decaying plant material, two interesting taxa were found with conidial morphologies that differed from those of any described species. One is described here as a new genus and species, *Dictyotrichocladium aquaticum*, and the other as a new species of *Minimelanolocus* R.F. Castañeda & Heredia, *M. aquatilis*.

Materials & methods

From November 2013 to August 2015, expeditions were made in Serra da Tromba (Chapada Diamantina, Bahia) and Serra da Fumaça (Pindobaçú, Bahia). Both areas belong to the semiarid region of northeastern Brazil.

In Serra da Tromba, submerged decaying leaves of *Calophyllum brasiliense* were collected from three streams in the Contas river basin, taken to the Laboratory of Mycology (LAMIC) in plastic bags, and processed as described by Bärlocher (2005). Leaf samples of *C. brasiliense* were washed, and 12 mm diam discs were cut with a cork borer. The discs were incubated for 48 hours at 18–20°C on a shaker (100 rpm) in 100 mL Erlenmeyer flasks containing 30 mL of sterile distilled water to induce sporulation. The spore suspensions were filtered through membrane filters (5 µm pore size), which were then mounted on slides with cotton blue in lactic acid. The leaf discs were mounted on slides with acid lactic and examined under compound microscope.

In Serra da Fumaça, submerged twigs were collected from some streams, taken to the Laboratory of Mycology (LAMIC) in plastic bags, and processed as described by Castañeda-Ruiz et al. (2016). The twigs were washed and placed in Petri dishes (moist chambers) incubated in a polystyrene box with sterile water plus glycerol for 30 days. The slides were mounted using resin PVL (polyvinyl alcohol, lactic acid and phenol). Measurements of reproductive structures and identification of specimens were performed using a compound microscope (Olympus BX51). The specimens were deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Dictyotrichocladium Fiuza, Gusmão & R.F. Castañeda, **gen. nov.**

MycoBank MB 821638

Differs from *Trichocladium* by its long clavate, turbinate to vermiform, curved or sigmoid, dictyoseptate conidia.

Type species: *Dictyotrichocladium aquaticum* Fiuza et al.

Etymology: Greek, *dictyo-*, for conidia with one or more transversal and longitudinal and/or oblique septa; Latin, *-trichocladium*, referring to the genus *Trichocladium*.

Conidiophores micronematous or macronematous, unbranched, septate, brown. Conidiogenous cells monoblastic, integrated, terminal, pale brown to brown. Conidial secession schizolytic. Conidia solitary, long pyriform, cylindrical, long clavate, turbinate to vermiform, curved or sigmoid, dictyoseptate, brown to dark brown.

Notes—*Dictyotrichocladium* is similar to *Trichocladium* Harz, which differs by its didymo- or phragmoconidia (Hughes 1952). Several other genera including *Acrodictys* M.B. Ellis, *Cirrenalia* Meyers & R.T. Moore, *Paradictyoarthrinium* Matsush., and *Thyrostroma* Höhn. have conidia similar to *Dictyotrichocladium*. However, *Acrodictys* produces cylindrical, pyriform, obovoid or ovoid and straight conidia (Zhao et al. 2011). *Cirrenalia* is close to *Dictyotrichocladium* in conidial shape but differs in its helicoid phragmoconidia (Zhao & Liu 2005). In *Paradictyoarthrinium*, the conidiophores are slightly constricted at the septa or monilioid, and conidiogenous cells are lateral or intercalary (Matsushima 1996, Seifert et al. 2011). *Thyrostroma* has stromatic, sporodochial conidiomata, and its conidiogenous cells are indeterminate with several enteroblastic percurrent generations (Höhnel 1911). *Dictyodesmium* S. Hughes and *Dictyorostrella* U. Braun also have dictyoseptate conidia, but both genera have stromatic, sporodochial conidiomata (Seifert et al. 2011).

Dictyotrichocladium aquaticum Fiuza, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 1

MycoBank MB821639

Differs from *Trichocladium* spp. by its long clavate to vermiform, curved or sigmoid, dictyoseptate conidia.

Type: Brazil, Bahia: Piatã, Chapada Diamantina, Serra da Tromba, 13°05′S 41°51′W, alt. 1272 m, on submerged decaying leaves of *Calophyllum brasiliense* Cambess. (*Calophyllaceae*) in a river, 5 June 2015, coll. P.O. Fiuza (**Holotype**, HUEFS 215954).

Etymology: Latin, *aquaticum,* referring to the aquatic environment where the species grows.

Colonies on natural substrate effuse, brown to dark brown. Mycelium superficial and immersed, composed of slightly branched, septate, smooth,

Fig. 1. *Dictyotrichocladium aquaticum* (holotype, HUEFS 215954). A–F. Conidia; G–I. Conidiophores, conidiogenous cells, and conidia. Scale bars = 20 µm.

pale brown to brown hyphae, 2–4.5 µm diam. Conidiophores micronematous or macronematous, single, erect, straight or flexuous, unbranched, 2–8-septate, smooth, 20–80 × 6–7.5 µm, brown. Conidiogenous cells monoblastic, integrated, terminal, smooth, 6–10 × 7–9 µm, pale brown to brown. Conidial secession schizolytic. Conidia solitary, long pyriform, cylindrical, long clavate, turbinate to vermiform, curved or sigmoid, dictyoseptate, smooth, 30–140 × 18–37.5 µm, brown to dark brown, dry.

Minimelanolocus aquatilis L.B. Conc., M.F.O. Marques, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 2

MycoBank MB821640

Differs from *Minimelanolocus limpidus* by its smaller conidiophores and verruculose conidia.

Type: Brazil, Bahia: Pindobaçú, Serra da Fumaça, 10°38′S 40°22′W, alt. 865 m, on submerged decaying twigs of unidentified plant in a stream, 8 August 2015, coll. L.B. Conceição (**Holotype**, HUEFS 216640).

Etymology: Latin, *aquatilis*, refers to its capacity or ability to live in water.

Colonies on natural substrate effuse, hairy, brown. Mycelium superficial and immersed, composed of slightly branched, septate, smooth, brown hyphae, 2–4 µm diam. Conidiophores macronematous, mononematous, single or caespitose, erect, straight or flexuous, unbranched, 1–5-septate, think-walled, smooth, 43–78 × 5–10 µm, brown to pale brown at the apex. Conidiogenous cells polyblastic, integrated, terminal, indeterminate, with several sympodial extensions, 20–42 × 5–7 µm, brown to pale brown. Conidial secession schizolytic. Conidia solitary, acropleurogenous, dry, 3–5-euseptate, broadly obclavate, verruculose, 21–30 × 5–8 µm, pale brown to subhyaline, with small dark scar at the truncate base, 1.5–2 µm wide.

Notes—*Minimelanolocus* currently comprises 29 species (Castañeda-Ruiz et al. 2001, Heredia et al. 2014, Liu et al. 2015, Hyde et al. 2016), most of which were found as saprobes on decaying leaves, rotten wood, dead branches, and bamboo in a terrestrial environment; only five species have been reported from aquatic habitats (Liu et al. 2015, Hyde et al. 2016). *Minimelanolocus aquatilis* is superficially similar to *M. limpidus* Heredia et al., which has smooth-walled and longer (27–48 µm) conidia (Heredia et al. 2014).

Discussion

Facultative aquatic hyphomycetes have been recorded in Brazil since 2011 (Barbosa et al. 2013). Thus far, 339 species are distributed in Amazon, Caatinga, and Atlantic Forest (Fiuza et al. 2016, 2017); of these, 25 were described as

Fig. 2. *Minimelanolocus aquatilis* (holotype, HUEFS 216640). A–F. Conidia; G–I. Conidiogenous cells; J. Conidiogenous cell and conidia; K, L. Conidiophores; M. Conidiophores, conidiogenous cells, and conidia. Scale bars: A–F, K–M = 10 µm; G–J = 5 µm.

new species: 15 from Amazon, 1 from Atlantic Forest, and 9 from Caatinga (Barbosa et al. 2011, 2013; Silva & Gusmão 2013; Monteiro & Gusmão 2014; Fiuza et al. 2015a,b, 2016; Moro et al. 2015; Conceição et al. 2016; Monteiro et al. 2016), including the type species of a new genus, *Dictyoaquaphila* J.S. Monteiro et al., from Amazon and Caatinga (Monteiro et al. 2016). The present study yielded another new species, *Minimelanolocus aquatilis* and a new genus, *Dictyotrichocladium*, to Caatinga.

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Lecanora shangrilaensis sp. nov., on pinecones from China

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Abstract—*Lecanora shangrilaensis* from southwestern China is described as a new species. It can be distinguished from other multispored species of *Lecanora* by the presence of usnic acid instead of atranorin and the epruinose discs with coarse granular epihymenium. It is otherwise characterized by the thin thallus, yellow to yellowish brown apothecial disc, 12–16-spored ascus, and the pinecone substrate.

Key words—East Asia, *Lecanoraceae*, lichenized *Ascomycota,* taxonomy

Introduction

Nine multispored species of *Lecanora* are known: *L. bruneri* Imshaug & Brodo, *L. cateilea* (Ach.) A. Massal., *L. japonica* Müll. Arg., *L. loekoesii* Y. Joshi et al., *L. subjaponica* L. Lü & H.Y. Wang, *L. weii* L.F. Han & S.Y. Guo, *L. pleospora* Müll. Arg., *L. praesistens* Nyl., and *L. strobilinoides* Giralt & Gómez-Bolea (Giralt & Gómez-Bolea 1991; Guderley & Lumbsch 1999; Han et al. 2009; Lü et al. 2011, 2012). A detailed key to these nine species was presented by Lü et al. (2012). Six species have been reported from China: *L. bruneri*, *L. cateilea*, *L. japonica*, *L. loekoesii*, *L. subjaponica*, and *L. weii* (Han et al. 2009; Lü et al. 2012; Wang et al. 2007, 2013).

A further study of specimens collected from China has revealed a new *Lecanora* species, described here as *L. shangrilaensis*.

Materials & methods

The specimens studied are housed in the Lichen Section, Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the Lichen Herbarium, Liaocheng University, Liaocheng, China (LCU-L).

Thalli were examined and measured under a COIC XTL7045B2 stereo-microscope and photographed with Olympus SZX16. The anatomical characters (e.g., apothecial sections, crystalline types, asci, ascospores) were examined by hand-cut sections under an Olympus CX41 polarizing microscope and photographed with an Olympus DP72 attached to a Olympus BX61 light microscope. The specimen was chemically analyzed using spot tests and thin layer chromatography (TLC) with solvent system C (Orange et al. 2010).

Taxonomy

Lecanora shangrilaensis Z.T. Zhao & L. Lü, **sp. nov.** PLATE 1

MycoBank MB812187

Differs from other multispored *Lecanora* spp. by its production of usnic acid instead of atranorin and its epruinose discs with coarse granular epihymenium.

TYPE: China. Yunnan Province, Shangrila, Tianshengqiao, alt. 3500 m, on cones of *Pinus* sp., 3 Nov. 2008, Wang 20083559 (**Holotype**, SDNU; **isotype**, LCU-L).

ETYMOLOGY: The epithet refers to the county where the species was collected.

THALLUS crustose; surface whitish to yellowish gray, thin, continuous, with yellowish verruca, esorediate; margin indistinct; prothallus not visible. APOTHECIA lecanorine, sessile to constricted at the base, 0.2–0.5 mm diam; disc yellow to yellowish brown, epruinose, plane to slightly concave; margin concolorous with thallus, thick, prominent, entire, smooth. AMPHITHECIUM with small crystals soluble in K; cortex distinct, with crystals soluble in K, basally not thickened, 30–50 μm thick; algal layer 20–35 μm thick; PARATHECIUM hyaline; EPIHYMENIUM brown to dark brown, pigment soluble in K, with coarse crystals soluble in K, 15–20 μm thick; HYMENIUM hyaline, 40–50 μm thick; HYPOTHECIUM hyaline, 20–45 μm thick; SUBHYMENIUM hyaline, 10–20 μm thick; PARAPHYSES simple, slightly thickened apically, ≤2.5 μm diam.; ASCI clavate, containing 12–16 spores; ASCOSPORES hyaline, simple, ellipsoid, 7.5–12.5 × 5–7.5 μm, wall <1 μm thick. PYCNIDIA not observed.

CHEMISTRY: cortex K–, C–, KC+ yellow, P–; medulla K–, C–, KC+ yellow, P–; usnic acid and fumarprotocetraric acid present.

ECOLOGY & DISTRIBUTION: The new species was found on pinecones from Tianshengqiao, Yunnan Province, southwestern China, at an altitude of 3500 m.

ADDITIONAL SPECIMENS EXAMINED: **CHINA. YUNNAN PROVINCE**, Shangrila, Tianshengqiao, alt. 3500 m, 3 Nov. 2008, H.Y Wang 20083505, 20083506 (SDNU).

REMARKS: *Lecanora shangrilaensis* is characterized by a thin thallus, yellowish discs, coarse granular epihymenium, 12–16-spored ascus, pinecone

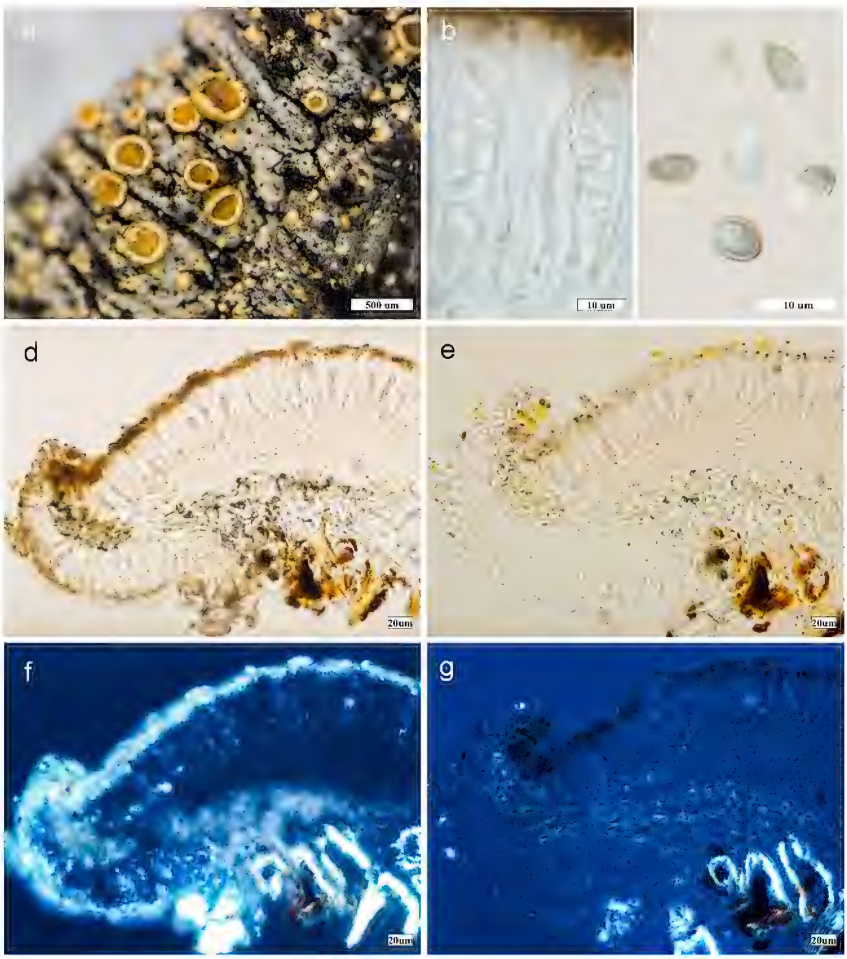

Plate 1. *Lecanora shangrilaensis* (holotype, SDNU 20083559). a, Thallus. b, Asci. c, Ascospores. d, Apothecium section. e, Pigment of epihymenium soluble in K. f, Crystals in apothecium section. g, Crystals of amphithecium and epihymenium soluble in K. Scale bars: a = 500 μm; b, c = 10 μm; d–g = 20 μm.

substrate, and presence of usnic and fumarprotocetraric acids. The most diagnostic character for recognizing the new taxon is the presence of usnic acid instead of atranorin. *Lecanora cateilea*, *L. loekoesii*, and *L. weii* resemble *L. shangrilaensis*, but differ in several characters: *L. cateilea* is distinguished by heavily pruinose apothecial discs, 8–12-spored asci, and possession of

psoromic acid and atranorin as secondary metabolites; *L. loekoesii* produces amphithecia with large crystals and atranorin and lacks phenolic compounds; and *L. weii* produces heavily pruinose apothecial discs and a *chlarotera*-type epihymenium. *Lecanora shangrilaensis* can be distinguished from other multispored species by the presence of usnic acid instead of atranorin and the yellowish apothecia. Morphologically the new species is similar to *L. polytropa* (Ehrh.) Rabenh., which also has a thin thallus and yellowish apothecial discs, but which has only 8 spores per ascus.

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Cephaliophora tropica: a third European record

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Abstract—We describe the morphology of *Cephaliophora tropica* based on a specimen retrieved from samples of urban water bodies in Łódź city, central Poland, and isolated by a shed snakeskin bait method. We also review the species' distribution to demonstrate its circumglobal range. With previous records from Hungary and Finland, this is only the third report of *C. tropica* from Europe.

Key words—aquatic environment, conidial fungi, distribution, hyphomycetes, *Pezizales*

Introduction

Cephaliophora Thaxt. (*Pezizales, Ascomycota*) encompasses hyphomycete species without known teleomorphs (Kirk et al. 2008). Thaxter (1903) originally erected the genus for two coprophilous species: *C. tropica* (type species) and *C. irregularis* Thaxt. [= *C. nigricans* (Bainier) Bainier]. A third species accepted in the genus is *C. uniformis* Narayanan, isolated from jute sacks (Narayanan 1962). Three rotifer- and tardigrade-capturing species were also described in *Cephaliophora* as *C. longispora* G.L. Barron et al., *C. muscicola* G.L. Barron et al., and *C. navicularis* Tzean & G.L. Barron (Tzean & Barron 1983, Barron et al, 1990), but it has been suggested that they belong in *Lecophagus* (Dick 1990, Tanabe et al. 1999).

Seifert et al. (2011) refer to *C. tropica* as a pantropical and occasionally temperate species. Our survey of literature citations of *C. tropica* indicates

Table 1. Literature review of *Cephaliophora tropica* reports

Country	Host/substrate *	Reference
Africa		
Central African Republic	Dung (*Bovidae*)	CBS 139.59
Egypt	Air-borne dust	Abdel-Hafez et al. (1990) Khallil & Abdel-Sater (1992)
	Moth pupae (*Noctuidae*)	Ismail & Abdel-Sater (1993)
Ghana	*Theobroma* (*Malvaceae*)	Farr & Rossman (2016)
Liberia	Dung (*Equidae*)	Thaxter (1903)
Asia		
China	Dung (*Muridae*)	Thaxter (1903)
	Endophytic (*Salicaceae*)	Zhao & Li (2012)
India	Dung (*Cercopithecidae*)	Iyer et al. (1973)
	Soil	Barron (1968)
Indonesia	Dung (*Muridae*)	Thaxter (1903)
Iran	Soil	Pordel et al. (2015)
Israel	*Trigonella* (*Fabaceae*)	Chabelska-Frydman (1959)
Japan	Vermicompost	Huang et al. 2013
Kuwait	Soil	Moustafa (1986) Al-Musallam (1987, 1990)
Pakistan	*Helianthus* (*Asteraceae*)	Shahzad & Ghaffar (1995) Sharfun-Nahar & Hashmi (2005) Mukhtar (2010) Mushtaq & Hashmi (2005)
	Ricinus (*Euphorbiaceae*)	Dawar et al. (2014)
	Diverse substrates	Ellis (1971)
Russia	Rhizosphere (*Sapindaceae*)	Zlotnikov et al. (2007)
Thailand	Dung (rodents)	Suwanarit (1986)
Turkey	*Capsicum* (*Solanaceae*)	CBS 996.69
Australia & Oceania		
Australia	Diverse substrates	Ellis (1971)
	Painted steel panels; organic materials	Crook & Hindson (1995)
Solomon Is.	Diverse substrates	Ellis (1971)
Europe		
Finland	Wooden parts (sauna)	Salonen & Ruokola (1969)
Hungary	Dung (*Cervidae*)	Tóth (1994) Révay (1998)
North & Central America		
Barbados	Soil	Barron (1968)
Jamaica	Dung (*Herpestidae*)	Thaxter (1903) Minter et al. (2001)
Mexico	*Vachellia* (*Fabaceae*)	Ale-Agha et al. (2007)
U.S.A.	*Roystonea* (*Arecaceae*) *Spathiphyllum* (*Araceae*)	Farr & Rossman (2016)
	Helianthus (*Asteraceae*)	Roberts et al. (1986)
	Nicotiana (*Solanaceae*)	Wolf (1949)
South America		
Venezuela	Dung (animals; incl. parrots)	Delgado Avila et al. (2002)

* Plant classification acc. to Stevens (2012)

its circumglobal distribution (Table 1), showing the most numerous data originating from Asia with no reports from Arctic and Antarctic. The only European records are from Finland (Salonen & Ruokola 1969) and Hungary (Révay 1998, and literature cited therein).

Materials & methods

In 2015, a study of fungal parasites of planktonic algae was conducted in urban, eutrophic aquatic reservoirs, including the artificial flow pond Młynek on the Olechówka river in Łódź, Central Poland (51°43′32″N 19°31′28″E). The river, which flows mainly within the city limits, also supplies several other artificial flow ponds. The Młynek pond (370 × 30–95 m) is part of an urban park system and in close proximity to a large residential area. The pond is used mainly for recreation, but no swimming is allowed.

On 21 July 2015 a water sample was collected from the shoreline of the pond and held in 125 ml plastic containers. Physical parameters measured included conductivity (EC = 483.1 µS/cm), pH (= 8.2), air temperature (= 25.8°C), and water temperature (= 25.7°C).

The sample was transported in an insulated container to the laboratory of the Department of Algology and Mycology, University of Łódź. A bait method was used in order to isolate fungi in the lab (Batko 1975). Three baits (sesame seeds, shed snakeskin, onion peels) were sterilized with hypochlorite solution and UV irradiation in a laminar-flow cabinet; they were distributed onto sterile Petri dishes together with 20 ml of water sample and incubated for 30 days at 24 ± 2°C. Fungi were identified according to Ellis (1971) with the aid of a Nikon SMZ 745T stereomicroscope and Nikon E-400 light microscope (semi-solid preparations) and photographed using a Nikon DS-F1 camera.

Taxonomy

Cephaliophora tropica Thaxt., Bot. Gaz. 35: 158 (1903) Plate 1

Colonies appearing light pink to reddish brown from the colour of conidial mass, effuse. Hyphae superficial, colourless, thin-walled, septate, 5.0–7.5 µm diam. Conidiophores macronematous, with terminal or rarely lateral ampulla, clavate, straight or slightly flexuous, colourless, smooth, 2–3-celled, 24–30 × 18–20 µm; basal cell trapezoidal, in 3-celled conidiophores the middle cell often slightly sloping. Conidiogenous cells terminal, subsphaerical to clavate, 19–29 × 16–20 µm, bearing up to 3 mature and tens of smaller immature conidia visible as balloon-like outgrowths at the surface of the conidiogenous cell. Conidia forming simultaneously, solitary, mostly 2–3-septate, 18–24 × 24–37 µm; cylindrical or slightly clavate, basal cell pointed, arising from a prominent wide (≤2.5 µm diam.) hilum; pale to cinnamon brown, basal and topical cells paler than the central ones, in some mature spores basal cell almost colourless; septa darker, brown, ≤2 µm thick.

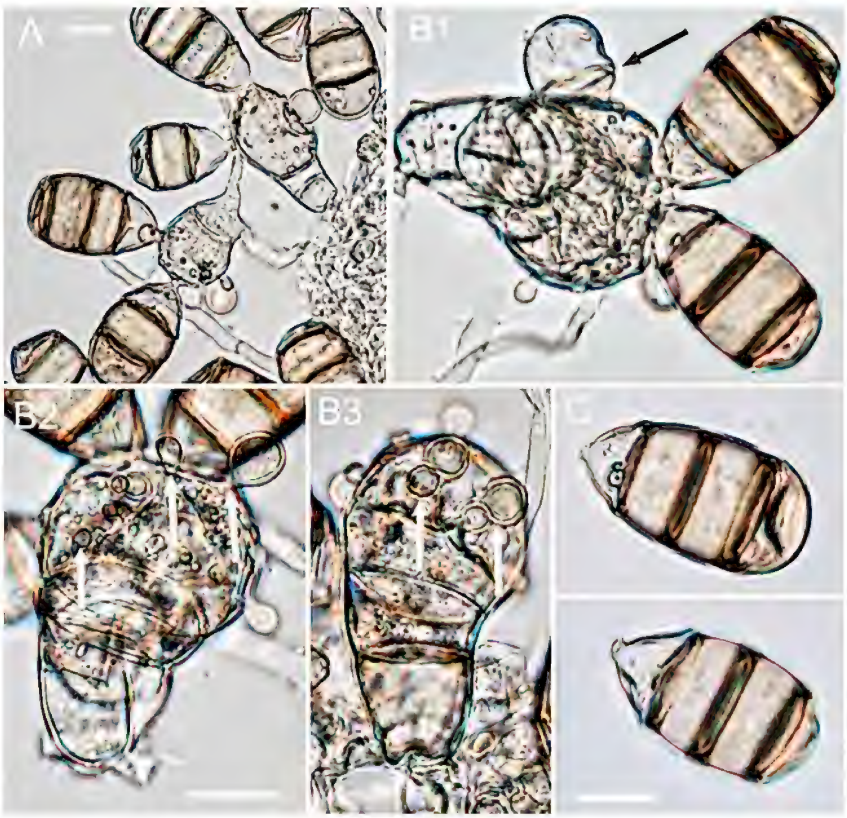

Plate 1. *Cephaliophora tropica* (LOD AF-33). A: Mature and immature conidiophores and conidia. B1–3: Conidiophores and conidiogenous cells; black arrow (B1) pointing to young two-celled conidium; white arrow (B2, B3) pointing to the youngest conidia visible as balloon-like outgrowths on the conidiogenous cell. C: mature conidia. Scale bars = 10 µm.

Specimen examined—**POLAND: Łódź city**, Młynek artificial flow pond, shore area, 21 July 2015, leg. P. Knysak (LOD AF-33).

Discussion

The size of the conidiophores and conidia reported by us match the lower limits of measurements given in other reports of the species. In conidia formed in the water-derived culture, the maximum number of septa was four. Similarly, Thaxter (1903) and Wolf (1949) reported 2–5-septate (mainly 3-septate) conidia, although subsequent cultural studies by Mayoral et al. (1964) and Weik & Pappelis (1964) noted conidia with up to 8 and 9 septa.

Although both of the latter studies reported conidia that were deformed or with atypical shapes, we have observed only regular conidial forms.

Beside the two main substrates of *C. tropica* (e.g., dung and soil), the diversity of reported substrata covers a range of living and dead plant and animal tissues (Table 1) as well as air-borne dust and a painted steel surface. To our knowledge, we are the first to isolate the species from a sample derived from an aquatic ecosystem. Although Kaushal & Singh (2012) reported *C. tropica* from areas surrounding water bodies in India, their photograph of the fungus does not contain necessary information to confirm the identification as *C. tropica*.

The ability to utilize diverse carbon and nitrogen sources was studied by Mayoral et al. (1964) and Weik & Pappelis (1964). *Cephaliophora tropica* grew well using all nine tested nitrogen-containing compounds, but fungal sporulation was enhanced by nitrate-N (Weik & Pappelis 1964). Cellobiose and hexoses (fructose, glucose, galactose, and mannose) proved to be the best carbon sources as indicated by mycelium dry weight and change of pH of cultural media (Mayoral et al. 1964). In our experiment, colonies of *C. tropica* developed only on shed snakeskin, suggesting that that substrate had a positive influence on growth. However, species preference toward keratin is still to be tested.

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Tretoheliocephala compacta gen. & sp. nov. from the Brazilian semi-arid region

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Abstract—A new genus and species, *Tretoheliocephala compacta*, found on decaying leaves of an unidentified plant, is described and illustrated. It is characterized by macronematous, erect dark brown conidiophores with a compact cluster of globose, monotretic conidiogenous cells, which produce solitary or blastocatenate, ovoid, oblong, dolabriform, sub-obpyriform or obcuneiform, 0–2-septate, brown conidia.

Key words—*Ascomycota*, hyphomycetes, taxonomy, tropics

Introduction

The diversity of conidial fungi in the Brazilian semi-arid region has received little attention. However, recent studies conducted in Caatinga biome reveal a high number of new microfungal species (Almeida et al. 2016, Conceição et al. 2016, Monteiro et al. 2016, Silva et al. 2016). During a survey of hyphomycetes associated with plant materials from Bahia state, a conspicuous fungus was collected that showed remarkable differences from all previously described genera (Seifert et al. 2011) and is described here as new.

Materials & methods

Samples of leaf litter were placed in plastic bags for transport to the laboratory, where they were placed in Petri dish moist chambers and stored at 25°C for 30 days in

a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol (Castañeda-Ruiz et al. 2016). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were made at ×1000 magnification. Micrographs were obtained with an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. The specimens, including the holotype, are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Tretoheliocephala Gusmão, J.S. Monteiro & R.F. Castañeda, **gen. nov.**

IndexFungorum IF553563

Differs from *Heliocephala* by its tretic conidial ontogeny.

Type species: *Tretoheliocephala compacta* Gusmão et al.

Etymology: Latin, *treto-*, referring to the tretic conidial ontogeny + *-heliocephala,* referring to the genus *Heliocephala.*

Conidial fungi. Colonies on the natural substratum effuse, hairy, dark brown or black. Conidiophores macronematous, mononematous, erect, unbranched or branched, cylindrical below, capitate at the apex, septate, with a distinct smooth or verruculose, brown or dark brown. Conidiogenous cells monotretic, discrete, globose, determinate, brown to dark brown, forming a more or less spherical cluster. Conidial secession schizolytic. Conidia solitary or blastocatenate, unicellular or euseptate, ovoid, oblong, dolabriform, subobpyriform or obcuneiform, smooth, pale brown or brown.

Tretoheliocephala compacta Gusmão, J.S. Monteiro & R.F. Castañeda, **sp. nov.**

Figs 1, 2

IndexFungorum IF553564

Differs from *Heliocephala* spp. by its monotretic conidiogenous cells.

Type: Brazil, Bahia: Mucugê, Parque Nacional da Chapada Diamantina, 13°00′S 41°22′W, on decaying of unidentified plant, 10 January 2005, coll. L.F.P. Gusmão (**Holotype**, HUEFS 81045).

Etymology: Latin, *compacta,* referring to the compact arrangement of conidiogenous cells in the capitulum.

Colonies on the natural substratum effuse, hairy, amphigenous, dark brown or black. Mycelium mostly immersed, composed of septate, smooth, brown hyphae, 1–2.5 µm diam. Conidiophores macronematous, mononematous, cylindrical below, capitate at the apex, erect, straight, robust, with 1–3 enteroblastic percurrent extension, 7–10-septate, unbranched or branched above, smooth below, somewhat verrucose or with remains of the old collapsed conidiogenous cells embedded near of each enteroblastic percurrent

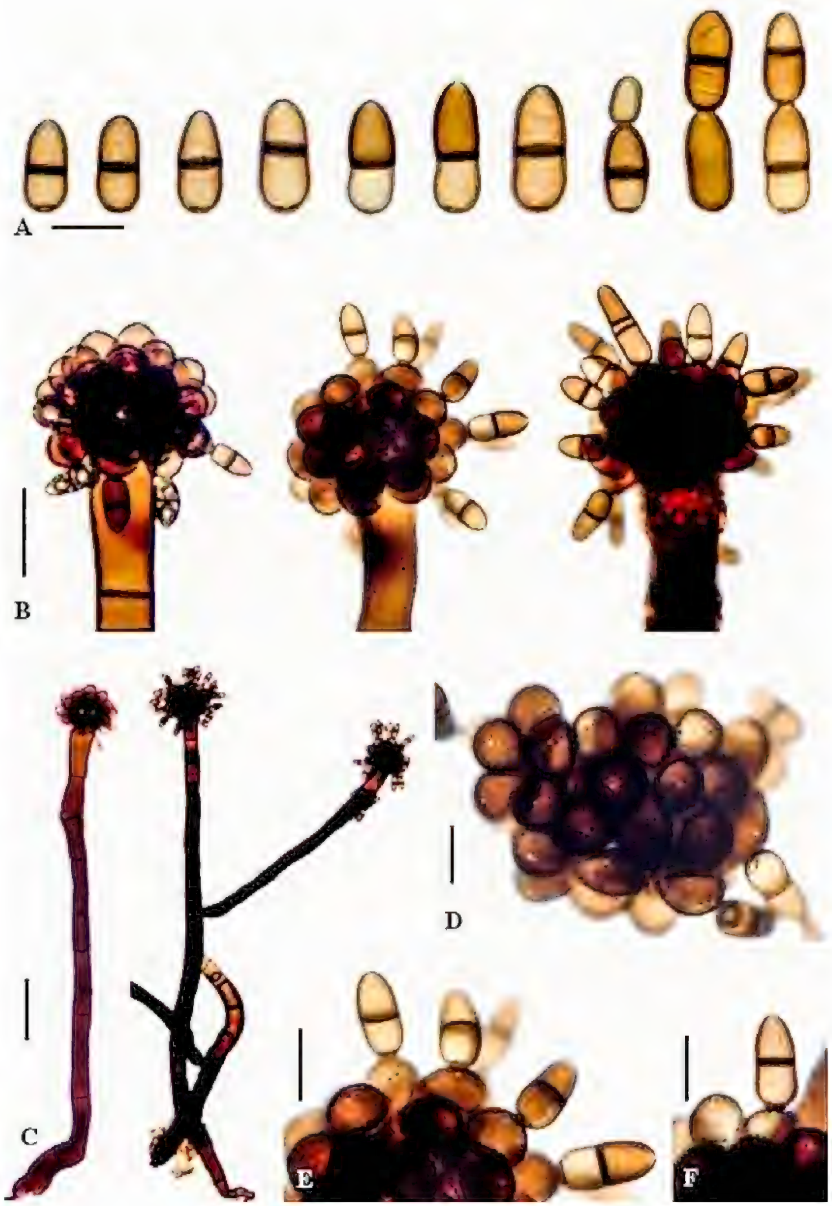

FIG. 1. *Tretoheliocephala compacta* (holotype, HUEFS 81045). A. Conidia. B. Clusters of conidiogenous cells and conidia. C. Conidiophores, conidiogenous cells, and conidia. D. Conidiogenous cells. E, F. Conidiogenous cells and conidia. Scale bars: A, D–F = 10 μm; B = 20 μm; C = 50 μm.

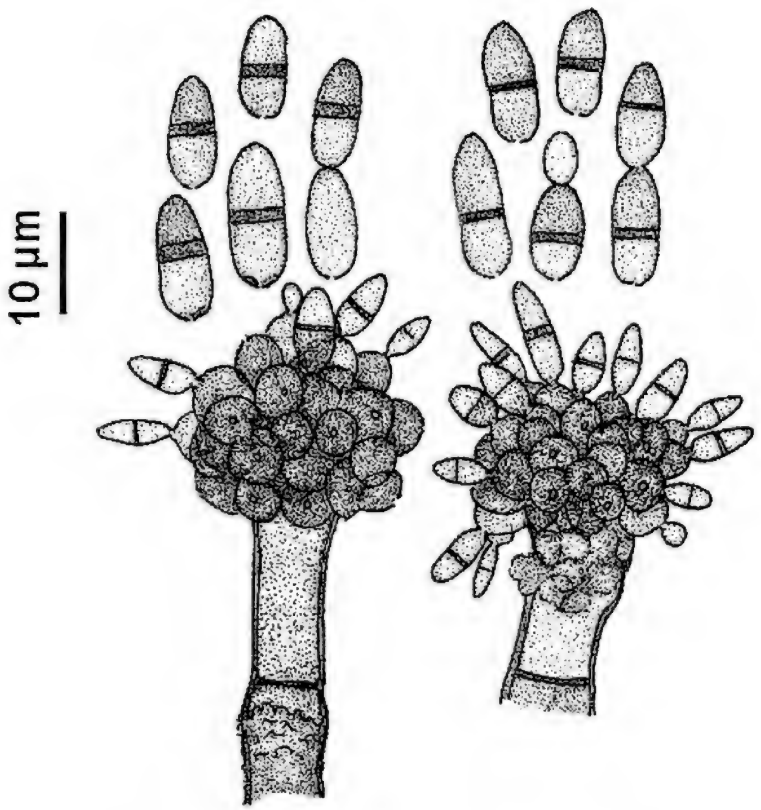

Fig. 2. *Tretoheliocephala compacta* (holotype, HUEFS 81045). Conidiogenous cells and conidia. Scale bar = 10 µm.

regeneration of the conidiophores, black below, dark brown or brown near the conidiogenous capitulum, 320–700 × 15–19 µm; branches black, 200–300 × 13–15 µm. Conidiogenous cells monotretic, discrete, globose, dark brown or brown, smooth, determinate, 8–12 µm diam, disposed in a compact cluster forming of the more or less spherical capitulum. Conidial secession schizolytic. Conidia solitary or blastocatenate, ovoid, oblong, dolabriform, subobpyriform or obcuneiform, 0–2-septate, mostly 1-septate, 12–15(–21) × 6–7 µm, pale brown, brown or dark brown, sometimes with the basal cell paler than the apical cell, black at septa.

Comments: *Heliocephala* V. Rao et al. resembles *Tretoheliocephala*, but *Heliocephala* is characterized by conidiophores that are macronematous, mononematous, brown, solitary, erect, straight or flexuous, with short branches and bearing terminal clusters of conidiogenous cells or elongated percurrently and bearing discrete clusters of conidiogenous cells along their lengths. *Heliocephala* also produces conidiogenous cells that are monoblastic, hyaline to light brown, ampulliform to lageniform borne on the short branches and conidia that are obclavate to navicular, subhyaline to olivaceous, 1–3-septate, rostrate, sometimes germinating at the rostrum to produce secondary clusters of conidiogenous cells (Decock et al. 1998, Heredia et al. 2011, Kumaresan & Srinivasan 2002, Mel'nik et al. 2013, Rao et al. 1984). These two genera are easily separated.

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Sporormiella longicolla sp. nov. and new *Sporormiella* records on herbivore dung from Brazil

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Abstract—Seven species of *Sporormiella* are reported from fresh dung collected in a survey including different vegetation areas throughout Pernambuco, Northeastern Brazil. An undescribed, long-necked, small-spored species is proposed as *Sporormiella longicolla*. *Sporormiella isomera* is a new record for the Neotropics; *S. australis*, *S. leporina*, and *S. pentamera* are new records for Brazil; and *S. herculea* (a rare species) and *S. minima* are also recorded. Descriptions, photographic plates and digital line drawings are provided, along with an identification key to all *Sporormiella* species recorded in Brazil. Substrate relationships and distribution data of these fungi are also discussed.

Key words—*Ascomycota*, coprophilous fungi, *Pleosporales, Sporormiaceae,* taxonomy

Introduction

Sporormiella Ellis & Everh. 1892 comprises ~80 pseudothecial species, usually with dark, 4-celled ascospores, commonly found on herbivore dung worldwide (Kirk et al. 2008). The genus is characterized by small, dark brown, glabrous or hairy uniloculate pseudothecia, bitunicate asci, cylindrical to clavate, bearing eight three–to many–septate, dark brown ascospores with conspicuous germ slits, surrounded by a hyaline gelatinous sheath (Ahmed & Cain 1972). Species of *Sporormiella* are predominantly coprophilous (Richardson 2001b), with occasional records as saprobes on

other substrata, and even as endophytes (Sun et al. 2006). Recently, the genus has received attention as a paleontological indicator to trace the wave of human invasion, habitat destruction, and subsequent megafaunal extinctions in Quaternary lake deposits (Comandini & Rinaldi 2004, Raper & Bush 2009).

The monophyly of *Sporormiella*, as well as other members of *Sporormiaceae* Munk, was studied by Kruys & Wedin (2009), who suggested a new generic classification of the family, in which *Preussia* Fuckel would include *Sporormiella* and *Sporormimula* Arx & Aa. Although Kruys & Wedin (2009) argued for the synonymy of *Preussia* and *Sporormiella*, they demonstrated no significant support for a joint monophyletic grouping and stated that resolution of the *Preussia–Sporormiella* complex required further study.

In Brazil, there have been few concentrated surveys on coprophilous mycobiota, and little is known about *Sporormiella*. After a visit to Brazil in 1998, Richardson (2001a) recorded *S. minima* and *S.* cf. *megalospora* (Auersw.) S.I. Ahmed & Cain in Bonito and Pantanal do Rio Negro, Mato Grosso do Sul State, providing short descriptions and discussing the importance of further investigations. Here, the *Sporormiella* species recorded on dung from Brazil are revisited, including revision of herbaria material, and new records and an undescribed species from fresh material collected in Pernambuco State.

Material & methods

Dung samples of camel (*Camelus bactrianus*), deer (*Cervus elaphus*), llama (*Lama glama*), and waterbuck (*Kobus ellipsiprymnus*) were collected from August 2009 to July 2010 in a zoological park in Recife (8°07′30″S 34°52′30″W), and samples of cattle (*Bos taurus*), goat (*Capra hircus*) and horse (*Equus caballus*) dung were collected from animal precincts on the campus of Universidade Federal Rural de Pernambuco, Recife (8°00′54″S 34°56′59″W), and from farms close to the Instituto Agronômico de Pernambuco (IPA) in Caruaru (8°01′59″S 36°06′59″W) and Serra Talhada (7°54′59″S 38°17′00″W), all located in Pernambuco, Northeastern Brazil. The samples were collected with sterilized spatulas, placed in clean plastic bags, taken to the laboratory in less than 24 hours and incubated in moist chambers at room temperature (28 ± 2 °C) for at least 60 days under alternating natural light and dark periods.

The specimens were observed directly from the substrata under a Leica EZ4 stereomicroscope. Pseudothecia were mounted in tap water and/or lactophenol with cotton blue for measurements and identification, and in Polyvinyl–Lacto–Glycerol resin for preservation. Specimens were identified based on morphology according to Ahmed & Cain (1972), Bell (1993, 2005), Richardson & Watling (1997), and

Doveri (2004). A survey of representative national collections of fungi (acronyms following Thiers 2016) was also conducted, including the herbaria Pe. Camille Torrend (URM), Dárdano de Andrade Lima (IPA), Maria Eneyda P. Kaufmann Fidalgo (SP), Dimitri Sucre Benjamin (RB), Instituto Nacional de Pesquisas da Amazônia (INPA), Museu Paraense Emilio Goeldi (MG), and Instituto de Biociências, Universidade Federal do Rio Grande do Sul (ICN). High quality images were captured with a QImaging QColor 3 digital camera mounted on an Olympus BX51 compound microscope using differential interference or phase contrast microscopy. Methods for the digital line drawing illustrations of both pseudothecia and microscopic structures were adapted from Barber & Keane (2007). Permanent slides were mounted and deposited in Herbário Padre Camille Torrend, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Sporormiella australis (Speg.) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 434 (1972) Fig. 1a–d

Pseudothecia scattered, immersed when young, becoming partially immersed to superficial on dung when mature, subglobose to obpyriform, dark brown to black, 185–210 × 145–175 µm diam. Neck small, papilliform, glabrous, black. Peridium membranaceous, semitransparent, composed of angular cells. Asci bitunicate, 8-spored, cylindrical to clavate, broader near the apex, rounded above, tapering abruptly downwards to form a short stipe, 125–140 × 14.5–18 µm. Ascospores obliquely triseriate, less commonly biseriate, 4-celled, cylindrical, rounded at the ends, light brown to brown, 37.5–40 × 6–7.5 µm, smooth, with easily separable segments in all septa, germ slits oblique, surrounded by a gelatinous sheath.

Material examined: **BRAZIL. Pernambuco**, Caruaru, Instituto Agronômico de Pernambuco (IPA), on cattle dung, 6.VI.2012, R.F.R. Melo (URM86773); Recife, Horto Zoobotânico do Parque Dois Irmãos, on goat dung, 12.IX.2011, R.F.R. Melo (URM86775).

Notes: *Sporormiella australis* can be easily mistaken for other common *Sporormiella* species based on ascospore shape, which are smaller in *S. minima* (27.5–30 × 4–5 µm) and larger in *S. intermedia* (Auersw.) S.I. Ahmed & Cain (48–59 × 9.5–11.5 µm) (Ahmed & Cain 1972). The ascospore cells of *S. australis* do not easily separate in all septa.

Sporormiella herculea (Ellis & Everh.) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 442 (1972) Fig. 1e–i

Pseudothecia scattered, immersed on dung, obpyriform, dark brown to black, 210–275 × 130–155 µm diam. Neck cylindrical, glabrous, black.

Peridium membranaceous, semitransparent to opaque, composed of angular cells. Asci bitunicate, 8-spored, cylindrical-clavate, with somewhat narrowed apex, tapering abruptly below to form a short stipe, 235–270 × 45–50 µm. Ascospores obliquely uni- to biseriate, 6–12-celled, cylindrical to fusiform, with a wide morphological diversity, rounded or narrowed at the ends, with some cells larger and thicker than the others, usually with the uppermost spore in the ascus bearing a greatly enlarged and pigmented cell, dark brown at maturity, 87.5–112 × 15–20(–25) µm, smooth, with transverse germ slits, surrounded by a gelatinous sheath.

Material examined: **BRAZIL. Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 9.I.2012, R.F.R. Melo s.n. (URM86777); Serra Talhada, Instituto Agronômico de Pernambuco, on horse dung, R.F.R Melo s.n. (URM86776).

Notes: *Sporormiella herculea* is distinguished by its 6–16-celled ascospores, with the second to fifth cell sometimes greatly enlarged in the uppermost spore in the ascus. Ahmed & Cain (1972) discussed morphological differences of Brazilian records.

Sporormiella isomera S.I. Ahmed & Cain, Canad. J. Bot. 50: 445 (1972) Fig. 1J–L

Pseudothecia scattered, immersed when young, becoming partially immersed on dung when mature, subglobose, dark brown to black, 190–245 µm in diam. Neck small, papilliform, glabrous, black. Peridium membranaceous, semitransparent, composed of angular cells. Asci 8-spored, cylindrical-clavate, broader near the apex, tapering gradually downwards to a short stalk, 130–150 × 12.5–15 µm. Ascospores obliquely biseriate, 4-celled, cylindrical, rounded at the ends, light brown when young, becoming dark brown at maturity, 35–40 × 5–7 µm, smooth, with easily separable segments in all septa, with germ slits parallel to oblique, with a crook near the middle, surrounded by a gelatinous sheath.

Material examined: **BRAZIL. Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on camel dung, 5.IV.2010, R.F.R. Melo s.n. (URM82397, 82398).

Notes: *Sporormiella isomera* is similar to *S. leporina*, except it possesses easily separable rounded terminal ascospore cells with a germ slit crooked near the middle. *Sporormiella minima* differs in having smaller ascospores (27.5–30 × 4–5 µm) and pseudothecia (115–165 × 90–125 µm) and asci that are abruptly constricted to form a stipe at their base.

Sporormiella leporina (Niessl) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 447 (1972) FIG. 1M,N

PSEUDOTHECIA isolated to scattered, immersed when young, becoming partially immersed on dung when mature, subglobose, dark brown to black, 180–195 × 150–170 µm. NECK cylindrical, glabrous, black. PERIDIUM membranaceous, semitransparent, composed of angular cells. ASCI 8-spored, cylindrical–clavate, with a short stalk, 95–125 × 12–17.5 µm. ASCOSPORES obliquely biseriate, 4-celled, cylindrical, narrowed at the ends, light brown when young, becoming dark brown at maturity, 30–36.5 × 5–6.5 µm, smooth, equally separable in all segments, with germ slits strongly oblique, surrounded by a gelatinous sheath.

MATERIAL EXAMINED: **BRAZIL. PERNAMBUCO**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on llama dung, 02.II.2010, R.F.R. Melo s.n. (URM82395, 82396).

NOTES: *Sporormiella leporina* can be distinguished by its cylindrical-clavate asci that are wider near the apex and gradually narrow toward the base and its ascospore cells with oblique germ slits.

Sporormiella longicolla R.F.R. Melo, **sp. nov.** FIGS 2, 3

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Differs from 4-celled *Sporormiella* species by its longer neck, longer and narrower asci, and ascospores with persistently united cells.

TYPE: Brazil. Pernambuco: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 29.II.2012, R.F.R. Melo s.n. (**Holotype**, URM87584).

ETYMOLOGY: *longicolla* = with a long neck.

PSEUDOTHECIA isolated to gregarious, immersed to semi–immersed on the substrate, subglobose to obpyriform, dark brown to black, 450–650 × 140–220 µm, glabrous. NECK cylindrical, long, glabrous, carbonaceous, black, 250–460(–550) × 40–65 µm, straight or flexuous. PERIDIUM pseudoparenchymatous, membranaceous, composed by angulated, thick–walled cells, 6–12 µm long. ASCI bitunicate, 8-spored, cylindrical-clavate, tapering slightly downwards to form a long stipe, twice the length of the sporiferous part, (72–)95–105(–137.5) × 7.5–10 µm. ASCOSPORES obliquely biseriate, 4-celled, slightly apiculate on both ends, hyaline when young, becoming brown to dark brown when mature, 17.5–20.5 × 3–3.5 µm, smooth, usually with the cells remaining united even after the spore liberation, with longitudinal germ slit, surrounded by a thin hyaline gelatinous sheath. Anamorph unknown.

ADDITIONAL MATERIAL EXAMINED: **BRAZIL. PERNAMBUCO**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 15.II.2012, R.F.R. Melo s.n. (URM87585).

Notes: *Sporormiella longicolla* has pseudothecia with long prominent necks, long slender asci with stipes c. 2× the length of the sporiferous part, and small stout ascospores with cells that remain united after spore liberation. This combination of morphological characters supports separation of a new species.

Sporormiella minima (Auersw.) S.I. Ahmed & Cain,
Pakistan J. Sci. Industr. Res. 12: 241 (1970) Fig. 1o–s

Pseudothecia isolated to gregarious, immersed to semi-immersed, subglobose to obpyriform, dark brown, 115–165 × 90–125 µm. Neck papilliform, glabrous, black, 45–50 × (35–)40–50 µm. Asci bitunicate, 8-spored, cylindrical, with the lower portion abruptly constricted to form a short stipe, 90–97.5 × 12.5–15 µm. Ascospores obliquely biseriate to triseriate, 4-celled, rounded at the ends, hyaline when young, becoming brown to dark brown, 27.5–30 × 4–5 µm, smooth, easily separable at the central septum, with germ slits parallel to the spore axis, with a crook at the central portion on each cell, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on llama dung, 13.X.2009, R.F.R. Melo (URM82389); 2.II.2010, R.F.R. Melo (URM82390); 1.III.2010, R.F.R. Melo (URM82391); 25.III.2010 (URM82392); 5.IV.2010 (URM82393); 2.VI.2010 (URM82394); on guinea pig dung, 1.XII.1947 (IPA37901).

Notes: *Sporormiella minima* is the most common *Sporormiella* species on herbivore dung from Brazil. Its striking features include the short ascus stipe formed by an abrupt end at its base, small ascospores (27.5–30 × 4–5 µm in the examined material) with a tendency to separate only at the central septum (even inside the asci), and parallel germ slits that form a distinct crook at the middle. Exsiccata IPA 37901 contained, along with the holotypus of *Ascobolus cuniculorum* Bat. & Pontual, several pseudothecia of *S. minima*, well preserved on dry guinea pig dung.

Sporormiella pentamera (Oudem.) S.I. Ahmed & Cain,
Canad. J. Bot. 50(3): 454 (1972) Fig 1t–v

Pseudothecia scattered to isolated, immersed to partially immersed on dung, subglobose, dark brown to black, 190–215.5 × 30–50 µm. Neck small, papilliform, glabrous, black. Asci 8-spored, clavate, with rounded apex, broader near the middle, tapering slightly downwards to form a short stipe, 190–210 × 15–17 µm. Ascospores biseriate, 5-celled, cylindrical, rounded to slightly narrowed at the ends, light brown when young, becoming dark

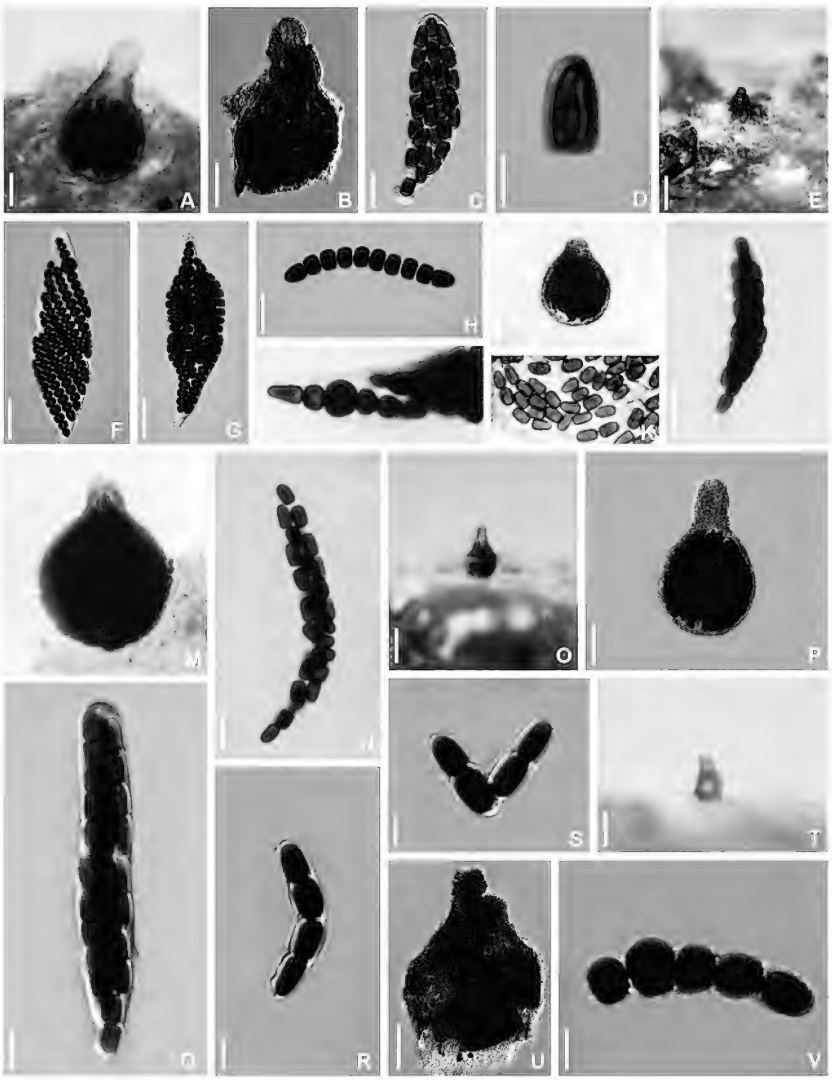

FIG. 1. *Sporormiella* species recorded on herbivore dung from Brazil. ***S. australis*** (URM86773): A. pseudothecium on dung; B. pseudothecium in mountant; C. mature ascus with cells united or equally separated on each septum; D. ascospore cell with a visible germ slit. ***S. herculea*** (URM86777): E. pseudothecium on dung; F, G. mature asci; H, I. ascospores. ***S. isomera*** (URM82398): J. pseudothecium in mountant; K. ascospores equally separated in all septa; L. mature ascus. ***S. leporina*** (URM82395): M. pseudothecium in mountant; N. mature ascus. ***S. minima*** (URM82391): O. pseudothecium on dung; P. pseudothecium in mountant; Q. mature ascus; R, S. ascospores, usually separated in the middle septum. ***S. pentamera*** (URM86786): T. pseudothecium on dung; U. pseudothecium in mountant; V. ascospores. Scale bars: A, B, F, G, U = 50 µm; C = 25 µm; D, R, S = 5 µm; E = 300 µm; H, I, L, M, P = 40 µm; J, O = 100 µm; K, Q = 10 µm; N, V = 15 µm; T = 150 µm.

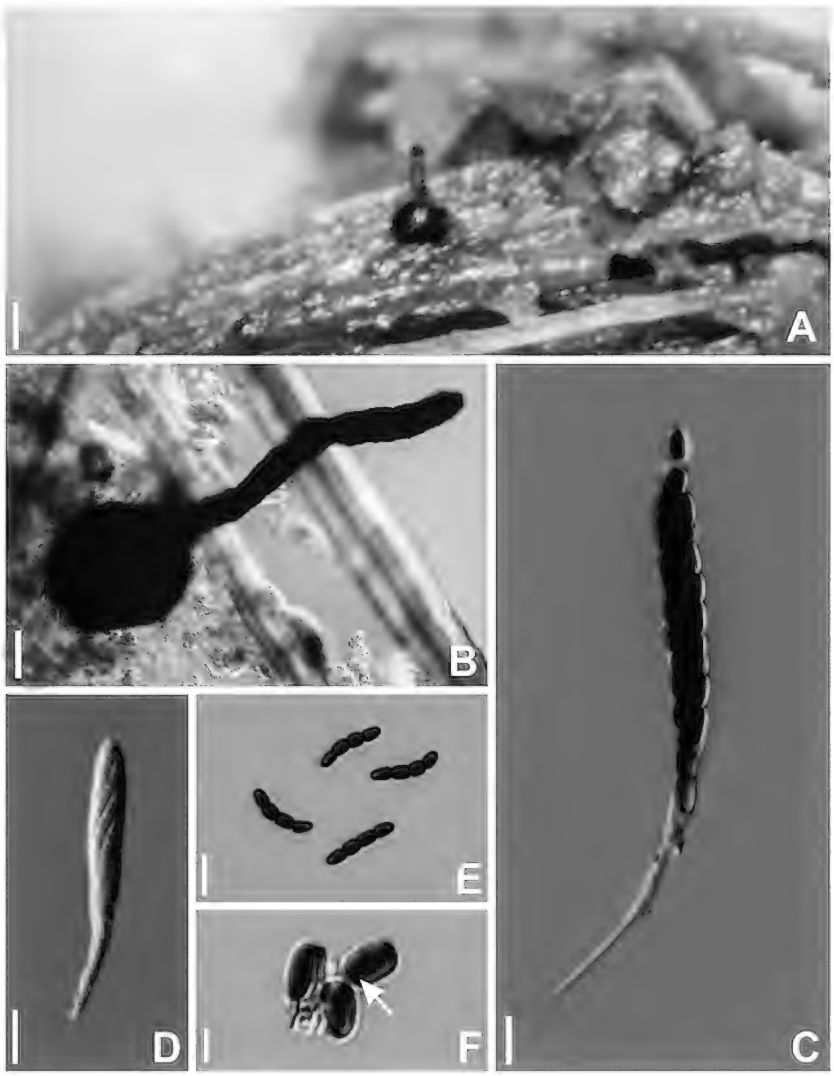

Fig. 2. ***Sporormiella longicolla*** (holotype, URM87584): A. pseudothecium on dung; B. pseudothecium in mountant; C. mature ascus; D. young ascus; E. ascospores with united cells even after their liberation; F. ascospore cells with visible germ slits (arrow). Scale bars: A = 300 µm; B = 60 µm; C = 7.5 µm; D = 15 µm; E = 10 µm; F = 2.5 µm.

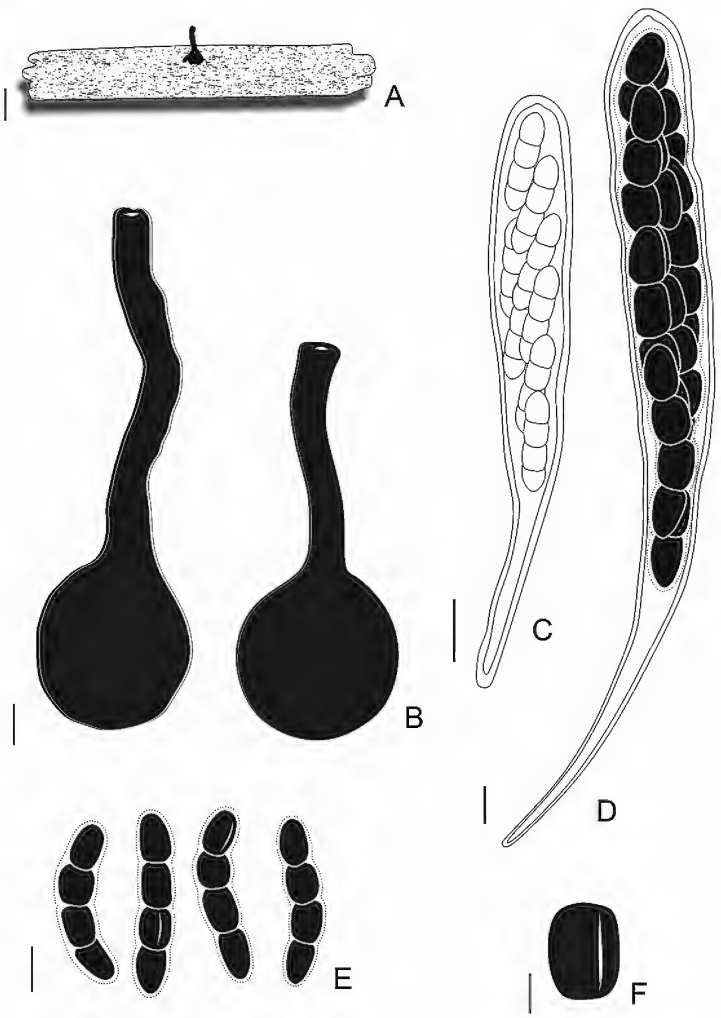

Fig. 3. ***Sporormiella longicolla*** (holotype, URM87584): A. pseudothecium on dung. B. long–necked pseudothecia. C. young ascus . D. mature ascus. E. ascospores with united cells even after their liberation. F. ascospore cell with a visible germ slit. Scale bars: A = 1 mm; B = 50 µm; C = 10 µm; D, E = 5 µm; F = 2 µm.

brown at maturity, 65–72.5 × 18–20.5 µm, usually with the second cell from the upper end of the spore larger than the others, smooth, with segments not easily separable, germ slits oblique, surrounded by a gelatinous sheath.

Material examined: **BRAZIL. Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 13.XI.2011, R.F.R. Melo (URM86786).

Key to species of *Sporormiella* on herbivore dung from Brazil

1. Ascospores 4-celled 2
   Ascospores >4-celled 3
2. Neck >250 µm long; ascospores 17.5–20.5 µm long ..... *S. longicolla*
   Neck <200 µm; ascospores >25 µm long 7
3. Asci tapering abruptly below to form a short stipe 4
   Asci tapering gradually from the broadest part into an elongated stipe 5
4. Ascospores 27.5–30 × 4–5 µm; germ slit parallel, forming a crook near the middle, with cells readily separable only at the central septum ..... *S. minima*
   Ascospores 37.5–40 × 6–7.5 µm; germ slit slightly to strongly oblique and cells easily separable at all segments ..... *S. australis*
5. Ascospores 80–90 × 19 µm; germ slits parallel to the long axis ..... *S.* cf. *megalospora*
   Ascospores shorter, <30 µm long; germ slits parallel to oblique 6
6. Germ slit parallel to oblique, with a crook near the middle ..... *S. isomera*
   Germ slit oblique, without a crook near the middle ..... *S. leporina*
7. Ascospores 5-celled ..... *S. pentamera*
   Ascospores 10–16-celled ..... *S. herculea*

Discussion

Due to their small size and frequent immersed habitat, *Sporormiella* pseudothecia can easily be overlooked in surveys of coprophilous fungi. Among the herbaria consulted, only two exsiccatae contained material collected in Brazil, one (IPA37901) representing a good specimen of *S. minima* and the other (URM1086) lacking a specific identification and not well preserved enough to allow accurate revision. Our study shows the flexibility regarding substrate preference and vegetation region by *Sporormiella* species. No clear substrate and/or regional preference could be detected. *Sporormiella minima,* with the highest number of records, was the most common *Sporormiella* species in herbivore dung in Brazil. These fungi usually specialize in exploiting herbivore dung and only occasionally have been recorded on other substrates due to stresses or disturbances affecting the community structure over the time.

According to Kruys & Wedin (2009), at one time *Sporormiella* circumscription was restricted to coprophilous species. Despite being controversial, the presence of the ostiole on the ascomata (traditionally used to separate *Preussia* from *Sporormiella*) appears to follow substrate preferences of species for both genera: *Preussia* species are non-ostiolate and found on plant debris, wood, or soil (Cain 1961), while *Sporormiella* species are ostiolate and coprophilous. This statement is supported by the polyphyly observed between the genera by Kruys & Wedin (2009) and by the inconsistent ostiole developmental pattern observed in *Sporormiaceae* by Guarro et al. (1997). The importance of active spore liberation is known in the study of coprophilous fungi (Ingold 1965, Webster 1970, Krug et al. 2004). However, development of the active method of liberation as an adaptation to coprophilous substrates in *Sporormiaceae* has yet to be studied.

Common species, like *S. minima* and *S. australis*, were found throughout the year alongside less common species. All exsiccatae containing representatives of *Sporormiella* in Herbarium Pe. Camille Torrand (URM) were collected in other countries under the name *Sporormia* and thus were not included in this treatment.

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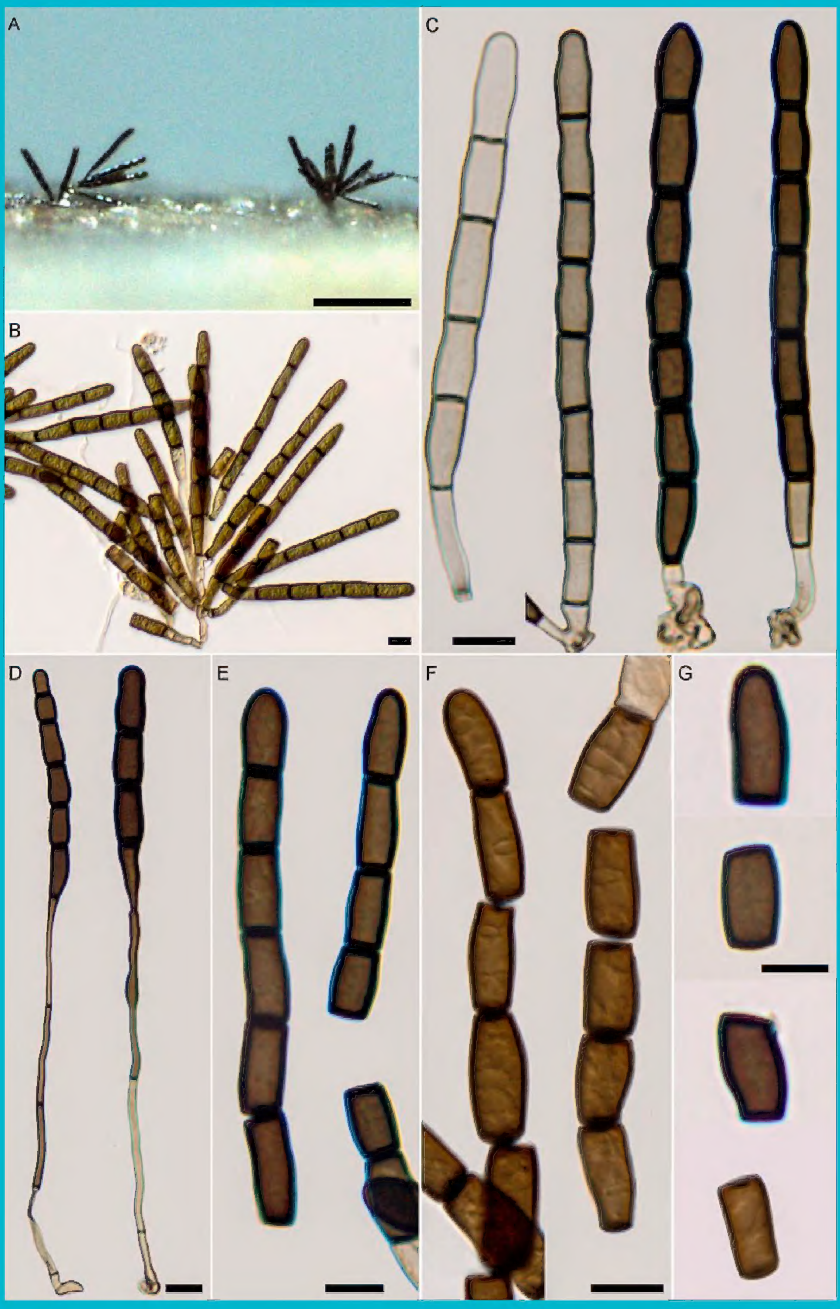

Arthrotaeniolella aquatica gen & sp. nov.
(Monteiro & al.— Fig. 1, p. 375)